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(54) Title: CYTOKINE RECEPTOR MOUSE ZCYTOR10

(57) Abstract

Novel polypeptides, polynucleotides encoding the polypeptides, and related compositions and methods are disclosed for mouse zcytor10, a novel mouse cytokine receptor. The polypeptides may be used within methods for detecting ligands that stimulate the proliferation and/or development of hematopoietic, lymphoid and myeloid cells. Ligand–binding receptor polypeptides can also be used to block ligand activity. The polynucleotides encoding mouse zcytor10 can be used to identify a human ortholog. The present invention also includes methods for producing the protein, uses therefor and antibodies thereto.

Description

CYTOKINE RECEPTOR MOUSE ZCYTOR10

BACKGROUND OF THE INVENTION

Hormones and polypeptide growth factors control proliferation and differentiation of cells of multicellular organisms. These diffusable molecules allow cells to communicate with each other and act in concert to form cells and organs, and to repair damaged tissue. Examples of hormones and growth factors include the steroid hormones (e.g. estrogen, testosterone), parathyroid hormone, follicle stimulating hormone, the interleukins, platelet derived growth factor (PDGF), epidermal growth factor (EGF), granulocyte-macrophage colony stimulating factor (GM-CSF), erythropoietin (EPO) and calcitonin.

Hormones and growth factors influence cellular metabolism by binding to receptors. Receptors may be integral membrane proteins that are linked to signaling pathways within the cell, such as second messenger systems. Other classes of receptors are soluble molecules, such as the transcription factors. Of particular interest are receptors for cytokines, molecules that promote the proliferation and/or differentiation of cells. Examples of cytokines include erythropoietin (EPO), which stimulates the development of red blood cells; thrombopoietin (TPO), which stimulates development of cells of the megakaryocyte lineage; and granulocyte-colony stimulating factor (G-CSF), which stimulates development of neutrophils. These cytokines are useful in restoring normal blood cell levels in patients suffering from anemia, thrombocytopenia, and neutropenia or receiving chemotherapy for cancer.

The demonstrated *in vivo* activities of these cytokines illustrate the enormous clinical potential of, and need for, other cytokines, cytokine agonists, and cytokine antagonists. The present invention addresses these needs by providing new a hematopoietic cytokine receptor, as well as related compositions and methods.

The present invention provides such polypeptides for these and other uses that should be apparent to those skilled in the art from the teachings herein.

DETAILED DESCRIPTION OF THE INVENTION

Prior to setting forth the invention in detail, it may be helpful to the understanding thereof to define the following terms:

The term "affinity tag" is used herein to denote a polypeptide segment that can be attached to a second polypeptide to provide for purification or detection of the second polypeptide or provide sites for attachment of the second polypeptide to a substrate. In principal, any peptide or protein for which an antibody or other specific binding agent is available can be used as an affinity tag. Affinity tags include a poly-histidine tract, protein A (Nilsson et al., EMBO J. 4:1075, 1985; Nilsson et al., Methods Enzymol. 198:3, 1991), glutathione S transferase (Smith and Johnson, Gene 67:31, 1988), Glu-Glu affinity tag (Grussenmeyer et al., Proc. Natl. Acad. Sci. USA 82:7952-4, 1985), substance P, Flag™ peptide (Hopp et al., Biotechnology 6:1204-10, 1988), streptavidin binding peptide, or other antigenic epitope or binding domain. See, in general, Ford et al., Protein Expression and Purification 2: 95-107, 1991. DNAs encoding affinity tags are available from commercial suppliers (e.g., Pharmacia Biotech, Piscataway, NJ).

The term "allelic variant" is used herein to denote any of two or more alternative forms of a gene occupying the same chromosomal locus. Allelic variation arises naturally through mutation, and may result in phenotypic polymorphism within populations. Gene mutations can be silent (no change in the encoded polypeptide) or may encode polypeptides having altered amino acid sequence. The term allelic variant is also used herein to denote a protein encoded by an allelic variant of a gene.

The terms "amino-terminal" and "carboxyl-terminal" are used herein to denote positions within polypeptides. Where the context allows, these terms are used with reference to a particular sequence or portion of a polypeptide to denote proximity or relative position. For example, a certain sequence positioned carboxyl-terminal to a reference sequence within a polypeptide is located proximal to the carboxyl terminus of

the reference sequence, but is not necessarily at the carboxyl terminus of the complete polypeptide.

The term "complement/anti-complement pair" denotes non-identical moieties that form a non-covalently associated, stable pair under appropriate conditions. For instance, biotin and avidin (or streptavidin) are prototypical members of a complement/anti-complement pair. Other exemplary complement/anti-complement pairs include receptor/ligand pairs, antibody/antigen (or hapten or epitope) pairs, sense/antisense polynucleotide pairs, and the like. Where subsequent dissociation of the complement/anti-complement pair is desirable, the complement/anti-complement pair preferably has a binding affinity of $<10^9\ M^{-1}$.

The term "complements of a polynucleotide molecule" is a polynucleotide molecule having a complementary base sequence and reverse orientation as compared to a reference sequence. For example, the sequence 5' ATGCACGGG 3' is complementary to 5' CCCGTGCAT 3'.

The term "contig" denotes a polynucleotide that has a contiguous stretch of identical or complementary sequence to another polynucleotide. Contiguous sequences are said to "overlap" a given stretch of polynucleotide sequence either in their entirety or along a partial stretch of the polynucleotide. For example, representative contigs to the polynucleotide sequence 5'-ATGGAGCTT-3' are 5'-AGCTTgagt-3' and 3'-tcgacTACC-5'.

The term "degenerate nucleotide sequence" denotes a sequence of nucleotides that includes one or more degenerate codons (as compared to a reference polynucleotide molecule that encodes a polypeptide). Degenerate codons contain different triplets of nucleotides, but encode the same amino acid residue (i.e., GAU and GAC triplets each encode Asp).

The term "expression vector" is used to denote a DNA molecule, linear or circular, that comprises a segment encoding a polypeptide of interest operably linked to additional segments that provide for its transcription. Such additional segments include promoter and terminator sequences, and may also include one or more origins of replication, one or more selectable markers, an enhancer, a polyadenylation signal,

etc. Expression vectors are generally derived from plasmid or viral DNA, or may contain elements of both.

The term "isolated", when applied to a polynucleotide, denotes that the polynucleotide has been removed from its natural genetic milieu and is thus free of other extraneous or unwanted coding sequences, and is in a form suitable for use within genetically engineered protein production systems. Such isolated molecules are those that are separated from their natural environment and include cDNA and genomic clones. Isolated DNA molecules of the present invention are free of other genes with which they are ordinarily associated, but may include naturally occurring 5' and 3' untranslated regions such as promoters and terminators. The identification of associated regions will be evident to one of ordinary skill in the art (see for example, Dynan and Tijan, Nature 316:774-78, 1985).

An "isolated" polypeptide or protein is a polypeptide or protein that is found in a condition other than its native environment, such as apart from blood and animal tissue. In a preferred form, the isolated polypeptide is substantially free of other polypeptides, particularly other polypeptides of animal origin. It is preferred to provide the polypeptides in a highly purified form, i.e. greater than 95% pure, more preferably greater than 99% pure. When used in this context, the term "isolated" does not exclude the presence of the same polypeptide in alternative physical forms, such as dimers or alternatively glycosylated or derivatized forms.

The term "operably linked", when referring to DNA segments, indicates that the segments are arranged so that they function in concert for their intended purposes, e.g., transcription initiates in the promoter and proceeds through the coding segment to the terminator.

The term "ortholog" denotes a polypeptide or protein obtained from one species that is the functional counterpart of a polypeptide or protein from a different species. Sequence differences among orthologs are the result of speciation.

"Paralogs" are distinct but structurally related proteins made by an organism. Paralogs are believed to arise through gene duplication. For example, α-globin, β-globin, and myoglobin are paralogs of each other.

A "polynucleotide" is a single- or double-stranded polymer of deoxyribonucleotide or ribonucleotide bases read from the 5' to the 3' end. Polynucleotides include RNA and DNA, and may be isolated from natural sources, synthesized *in vitro*, or prepared from a combination of natural and synthetic molecules. Sizes of polynucleotides are expressed as base pairs (abbreviated "bp"), nucleotides ("nt"), or kilobases ("kb"). Where the context allows, the latter two terms may describe polynucleotides that are single-stranded or double-stranded. When the term is applied to double-stranded molecules it is used to denote overall length and will be understood to be equivalent to the term "base pairs". It will be recognized by those skilled in the art that the two strands of a double-stranded polynucleotide may differ slightly in length and that the ends thereof may be staggered as a result of enzymatic cleavage; thus all nucleotides within a double-stranded polynucleotide molecule may not be paired.

A "polypeptide" is a polymer of amino acid residues joined by peptide bonds, whether produced naturally or synthetically. Polypeptides of less than about 10 amino acid residues are commonly referred to as "peptides".

The term "promoter" is used herein for its art-recognized meaning to denote a portion of a gene containing DNA sequences that provide for the binding of RNA polymerase and initiation of transcription. Promoter sequences are commonly, but not always, found in the 5' non-coding regions of genes.

A "protein" is a macromolecule comprising one or more polypeptide chains. A protein may also comprise non-peptidic components, such as carbohydrate groups. Carbohydrates and other non-peptidic substituents may be added to a protein by the cell in which the protein is produced, and will vary with the type of cell. Proteins are defined herein in terms of their amino acid backbone structures; substituents such as carbohydrate groups are generally not specified, but may be present nonetheless.

The term "receptor" is used herein to denote a cell-associated protein, or a polypeptide subunit of such a protein, that binds to a bioactive molecule (the "ligand") and mediates the effect of the ligand on the cell. Binding of ligand to receptor results in a conformational change in the receptor (and, in some cases, receptor multimerization, i.e., association of identical or different receptor subunits) that causes interactions

between the effector domain(s) and other molecule(s) in the cell. These interactions in turn lead to alterations in the metabolism of the cell. Metabolic events that are linked to receptor-ligand interactions include gene transcription, phosphorylation, dephosphorylation, cell proliferation, increases in cyclic AMP production, mobilization of cellular calcium, mobilization of membrane lipids, cell adhesion, hydrolysis of inositol lipids and hydrolysis of phospholipids. Cell-surface cytokine receptors are characterized by a multi-domain structure as discussed in more detail below. These receptors are anchored in the cell membrane by a transmembrane domain characterized by a sequence of hydrophobic amino acid residues (typically about 21-25 residues), which is commonly flanked by positively charged residues (Lys or Arg). In general, receptors can be membrane bound, cytosolic or nuclear; monomeric (e.g., thyroid stimulating hormone receptor, beta-adrenergic receptor) or multimeric (e.g., PDGF receptor, growth hormone receptor, IL-3 receptor, GM-CSF receptor, G-CSF receptor, erythropoietin receptor and IL-6 receptor). The term "receptor polypeptide" is used to denote complete receptor polypeptide chains and portions thereof, including isolated functional domains (e.g., ligand-binding domains).

A "secretory signal sequence" is a DNA sequence that encodes a polypeptide (a "secretory peptide") that, as a component of a larger polypeptide, directs the larger polypeptide through a secretory pathway of a cell in which it is synthesized. The larger peptide is commonly cleaved to remove the secretory peptide during transit through the secretory pathway.

A "soluble receptor" is a receptor polypeptide that is not bound to a cell membrane. Soluble receptors are most commonly ligand-binding receptor polypeptides that lack transmembrane and cytoplasmic domains. Soluble receptors can comprise additional amino acid residues, such as affinity tags that provide for purification of the polypeptide or provide sites for attachment of the polypeptide to a substrate, or immunoglobulin constant region sequences. Many cell-surface receptors have naturally occurring, soluble counterparts that are produced by proteolysis. Soluble receptor polypeptides are said to be substantially free of transmembrane and intracellular polypeptide segments when they lack sufficient portions of these segments to provide membrane anchoring or signal transduction, respectively.

The term "splice variant" is used herein to denote alternative forms of RNA transcribed from a gene. Splice variation arises naturally through use of alternative splicing sites within a transcribed RNA molecule, or less commonly between separately transcribed RNA molecules, and may result in several mRNAs transcribed from the same gene. Splice variants may encode polypeptides having altered amino acid sequence. The term splice variant is also used herein to denote a protein encoded by a splice variant of an mRNA transcribed from a gene.

Molecular weights and lengths of polymers determined by imprecise analytical methods (e.g., gel electrophoresis) will be understood to be approximate values. When such a value is expressed as "about" X or "approximately" X, the stated value of X will be understood to be accurate to ±10%.

All references cited herein are incorporated by reference in their entirety.

The present invention is based in part upon the discovery of a novel murine DNA sequence that encodes a protein having the structure of a class I cytokine receptor. The deduced amino acid sequence indicated that the encoded receptor belongs to the receptor subfamily that includes the EPO receptor. The polypeptide has been designated mouse zcytor10.

The novel mouse zcytor10 polypeptides of the present invention were initially identified by querying an EST database. An EST was found and its corresponding cDNA was sequenced. The novel polypeptide encoded by the cDNA showed homology with class I cytokine receptors. The mouse zcytor10 polynucleotide sequence encodes the entire coding sequence of the predicted protein. Mouse zcytor10 is a novel cytokine receptor that may be involved in cell proliferation or differentiation, an apoptotic cellular pathway, cell-cell signaling molecule, growth factor receptor, or extracellular matrix associated protein with growth factor hormone activity, or the like.

The sequence of the mouse zcytor10 polypeptide was deduced from a single clone that contained its corresponding polynucleotide sequence. The clone was obtained from murine embryo and placenta libraries. Other libraries that might also be searched for such sequences include PBL, thymus, spleen, lymph node, human

erythroleukemia cell lines (e.g., TF-1), Raji cells, acute monocytic leukemia cell lines, other lymphoid and hematopoietic cell lines, and the like.

The nucleotide sequence of a representative mouse zcytor10-encoding DNA is described in SEQ ID NO:1 (from nucleotide 215 to 1285), and its deduced 357 amino acid sequence is described in SEQ ID NO:2. An alternatively spliced mouse zcytor10-encoding DNA is described in SEQ ID NO:34 (from nucleotide 74 to 1151), and its deduced 359 amino acid sequence is described in SEQ ID NO:35. In its entirety, the mouse zcytor10 polypeptide represents a full-length polypeptide segment (residue 1 (Met) to residue 357 (Leu) of SEQ ID NO:2; or alternatively residue 1 (Met) to residue 359 (Leu) of SEQ ID NO:35). The domains and structural features of the mouse zcytor10 polypeptide are further described below.

Analysis of the mouse zcytor10 polypeptide encoded by the DNA sequence of SEQ ID NO:1 revealed an open reading frame encoding 357 amino acids (SEQ ID NO:2) comprising a predicted secretory signal peptide of 14 amino acid residues (residue 1 (Met) to residue 14 (Gly) of SEQ ID NO:2), and a mature polypeptide of 343 amino acids (residue 15 (Cys) to residue 357 (Leu) of SEQ ID NO:2). In addition, a motif having structural and functional similarity to the WSXWS motif (SEQ ID NO:3), hereinafter referred to as the "WSXWS-like motif," is present in mouse zcytor10 and corresponds to residues 199 to 203 of SEQ ID NO:2. The mouse zcytor10 receptor further comprises a cytokine-binding domain of approximately 200 amino acid residues (residues 15 (Cys) to 230 (Pro) of SEQ ID NO:2); a domain linker (residues 114 (Lys) to 121 (Val) of SEQ ID NO:2); a penultimate strand region (residues 177 (Ala) to 185 (Arg) of SEQ ID NO:2); a transmembrane domain (residues 231 (Leu) to 251 (Leu) of SEQ ID NO:2); complete intracellular signaling domain (residues 252 (Arg) to 357 (Leu) of SEQ ID NO:2) which contains a "Box I" signaling site (residues 260 (Leu) to 267 (Pro) of SEQ ID NO:2), and a "Box II" signaling site (residues 298 (Thr) to 302 (Asp) of SEQ ID NO:2). Those skilled in the art will recognize that these domain boundaries are approximate, and are based on alignments with known proteins and predictions of protein folding. In addition to these domains, conserved receptor features in the encoded receptor include (as shown in SEQ ID NO:2) a conserved Trp residue at positions 135 and 159, and a conserved Arg residue at

position 185. The corresponding polynucleotides encoding the mouse zcytor10 polypeptide regions, domains, motifs, residues and sequences described above are as shown in SEQ ID NO:1.

Analysis of the DNA sequence of SEQ ID NO:1 also revealed a potential intron sequence, which if spliced out of the message revealed an alternative zcytor10 polypeptide sequence. A potential intron lies between a splice donor site at the G and T nucleotides at numbers 153-154 in SEQ ID NO:1 and splice acceptor at A and G nucleotides at numbers 287-288 in SEQ ID NO:1. When spliced, an alternative form of zcytor10 cDNA results, as shown in SEQ ID NO:34. The corresponding splice variant zcytor10 polypeptide is shown in SEQ ID NO:35. Analysis of SEQ ID NO:35 showed a class I cytokine receptor polypeptide encoding 359 amino acids (SEQ ID NO:35) comprising a predicted secretory signal peptide of 16 amino acid residues (residue 1 (Met) to residue 16 (Ala) of SEQ ID NO:35), and a mature polypeptide of 343 amino acids (residue 17 (Ala) to residue 359 (Leu) of SEQ ID NO:35). The alternative form of the mouse zcytor10 receptor has all the features as described above for SEQ ID NO:2 including a "WSXWS-like motif," corresponding to residues 201 to 205 of SEQ ID NO:35. The mouse zcytor10 receptor further comprises a cytokine-binding domain of approximately 200 amino acid residues (residues 17 (Ala) to 232 (Pro) of SEQ ID NO:35); a domain linker (residues 116 (Lys) to 123 (Val) of SEQ ID NO:35); a penultimate strand region (residues 179 (Ala) to 187 (Arg) of SEQ ID NO:35); a transmembrane domain (residues 233 (Leu) to 253 (Leu) of SEQ ID NO:35); complete intracellular signaling domain (residues 254 (Arg) to 359 (Leu) of SEQ ID NO:35) which contains a "Box I" signaling site (residues 262 (Leu) to 269 (Pro) of SEQ ID NO:35), and a "Box II" signaling site (residues 300 (Thr) to 304 (Asp) of SEQ ID NO:35). Those skilled in the art will recognize that these domain boundaries are approximate, and are based on alignments with known proteins and predictions of protein folding. In addition to these domains, conserved receptor features in the encoded receptor include (as shown in SEQ ID NO:35) a conserved Trp residue at positions 137 and 161, and a conserved Arg residue at position 187. The corresponding polynucleotides encoding the mouse zcytor10 polypeptide regions, domains, motifs, residues and sequences described above are as shown in SEQ ID NO:34.

In the CXW site at the N terminal end of murine zcytor10 (Val-Thr-Trp; amino acid residues 39 (Val) to 41 (Trp) of SEQ ID NO:2; amino acid residues 41 (Val) to 43 (Trp) of SEQ ID NO:35) the cysteine residue is absent, and replaced by a valine. This is an unusual modification of the site, however there remain an even number of cysteines in the extracellular domain, leaving no unpaired cysteines and hence retention of the zcytor10 cytokine receptor three-dimensional structure. Moreover, the sequence of the murine zcytor10 WSXWS-like motif is unusual in that it contains a proline in the position of the first tryptophan and a threonine at the location of the second serine: PSWET (SEQ ID NO:40). This WSXWS-like motif similar to that of the IL-3Rα and IL-3Rβ subunits. IL-3Rβ is a common beta subunit, that heterodimerizes with IL-3Rα when binding it's ligand IL-3; however, the IL-3Rβ subunit heterodimerizes with GM-CSF receptor subunit when binding the ligand, GM-CSF. Similarly, the zcytor10, receptor which has similarities to the IL-3R and IL-2Rγ common subunit (discussed herein), can form a heterodimer and bind a variety of cytokine receptor subunits, and similarly transduce signals from different ligands. The zcytor10 Box I and Box II sites contain fairly typical sequences for this receptor family.

Moreover, within the intracellular signaling domain, there is a region of the zcytor10 sequence from approximately the Box I signaling site to near the start of Box II signaling site which comprises a highly conserved mammalian signaling motif comprising SEQ ID NO:41 that contains a Box I mammalian consensus sequence of SEQ ID NO:42. The conservation within the signaling motif, and Box I suggests that this region of zcytor10 has functional significance, and it is therefore preferable to maintain the conserved residues within this region and the Box I consensus in designing fusions within the intracellular signaling domain, or making variant zcytor10 polypeptides.

In addition there are other conserved motifs throughout zcytor10. Multiple alignment of zcytor10 with other members of the protein family revealed the following regions and motifs of conserved amino acids within the extracellular binding domain:

1) The first region, referred to hereinafter as "block 1," corresponds to amino acid residues 25 (Gly) to amino acid residue 230 (Pro) of SEQ ID NO:2. Block

1 defines a common extracellular cytokine binding domain between the variant forms of zcytor10 (SEQ ID NO:2 and SEQ ID NO:35).

2) Within block 1 there are several conserved motifs. The first motif, referred to hereinafter as "Motif 1," is described in SEQ ID NO:43, and corresponds to amino acid residues 34 (Leu) to amino acid residue 41 (Trp) of SEQ ID NO:2.

The second motif, referred to hereinafter as "Motif 2," is described in SEQ ID NO:44, and corresponds to amino acid residues 77 (Thr) to amino acid residue 80 (Cys) of SEQ ID NO:2.

The third motif, referred to hereinafter as "Motif 3," is Leu-Lys-Pro (LKP), and corresponds to amino acid residues 113 (Leu) to amino acid residue 115 (Pro) of SEQ ID NO:2.

The fourth motif, referred to hereinafter as "Motif 4," is Val-Thr-Val (VTV), and corresponds to amino acid residues 131 (Val) to amino acid residue 133 (Val) of SEQ ID NO:2.

The fifth motif, referred to hereinafter as "Motif 5," is described in SEQ ID NO:45, and corresponds to amino acid residues 145 (Tyr) to amino acid residue 148 (Gln) of SEQ ID NO:2.

The sixth motif, referred to hereinafter as "Motif 6," is Gly-Leu-Asp (GLD), and corresponds to amino acid residues 173 (Gly) to amino acid residue 173 (Asp) of SEQ ID NO:2.

The conservation of motifs 1 through 6 suggests that these motifs within zcytor10 have structural or functional significance, and it is therefore preferable to maintain these conserved motifs within the extracellular cytokine-binding domain in designing fusions within the extracellular binding domain, or in making variant zcytor10 polypeptides.

Motifs 1 through 6 are spaced apart from N-terminus to C-terminus, within the extracellular binding domain, in a configuration represented by the following:

M1-{32-35}-M2-{31-32}-M3-{14-15}-M4-{11}-M5-{22-24}-M6,

where M# denotes the specific motif disclosed above and

{#} denotes the number of amino acids between the motifs.

The presence of transmembrane regions, and conserved and low variance motifs generally correlates with or defines important structural regions in proteins. Regions of low variance (e.g., hydrophobic clusters) are generally present in regions of structural importance (Sheppard, P. et al., supra.). Such regions of low variance often contain rare or infrequent amino acids, such as Tryptophan. The regions flanking and between such conserved and low variance motifs may be more variable, but are often functionally significant because they may relate to or define important structures and activities such as binding domains, biological and enzymatic activity, signal transduction, cell-cell interaction, tissue localization domains and the like.

The regions of conserved amino acid residues in mouse zcytor10, described above, can be used as tools to identify new family members. For instance, reverse transcription-polymerase chain reaction (RT-PCR) can be used to amplify sequences encoding the conserved regions from RNA obtained from a variety of tissue sources or cell lines. In particular, highly degenerate primers designed from the mouse zcytor10 sequences are useful for this purpose. Designing and using such degenerate primers may be readily performed by one of skill in the art.

The present invention provides polynucleotide molecules, including DNA and RNA molecules, that encode the mouse zcytor10 polypeptides disclosed herein. Those skilled in the art will recognize that, in view of the degeneracy of the genetic code, considerable sequence variation is possible among these polynucleotide molecules. SEQ ID NO:4 and SEQ ID NO:39 are a degenerate DNA sequences that encompass all DNAs that encode the mouse zcytor10 polypeptide of SEQ ID NO:2 and SEQ ID NO:35 respectively. Those skilled in the art will recognize that the degenerate sequence of SEQ ID NO:4 and SEQ ID NO:39 also provide all RNA sequences encoding SEQ ID NO:2 and SEQ ID NO:35 respectively by substituting U for T. Thus, mouse zcytor10 polypeptide-encoding polynucleotides comprising nucleotide 1 to nucleotide 1071 of SEQ ID NO:4 and nucleotide 1 to nucleotide 1077 of SEQ ID NO:39 and their RNA equivalents are contemplated by the present invention. Table 1 sets forth the one-letter codes used

within SEQ ID NO:4 and SEQ ID NO:39 to denote degenerate nucleotide positions. "Resolutions" are the nucleotides denoted by a code letter. "Complement" indicates the code for the complementary nucleotide(s). For example, the code Y denotes either C or T, and its complement R denotes A or G, A being complementary to T, and G being complementary to C.

TABLE 1

Nucleotide	Resolution	Complement	Resolution
A	A	T	T
C	C	G	G
G	G	C	C
T	T	A	A
R	A|G	Y	C|T
Y	C|T	R	A|G
M	A|C	K	G|T
K	G|T	M	A|C
S	C|G	S	C|G
W	A|T	W	A|T
H	A|C|T	D	A|G|T
B	C|G|T	V	A|C|G
V	A|C|G	B	C|G|T
D	A|G|T	H	A|C|T
N	A|C|G|T	N	A|C|G|T

The degenerate codons used in SEQ ID NO:4 and SEQ ID NO:39, encompassing all possible codons for a given amino acid, are set forth in Table 2.

TABLE 2

Amino Acid	One Letter Code	Codons	Degenerate Codon
Cys	C	TGC TGT	TGY
Ser	S	AGC AGT TCA TCC TCG TCT	WSN
Thr	T	ACA ACC ACG ACT	ACN
Pro	P	CCA CCC CCG CCT	CCN
Ala	A	GCA GCC GCG GCT	GCN
Gly	G	GGA GGC GGG GGT	GGN
Asn	N	AAC AAT	AAY
Asp	D	GAC GAT	GAY
Glu	E	GAA GAG	GAR
Gln	Q	CAA CAG	CAR
His	H	CAC CAT	CAY
Arg	R	AGA AGG CGA CGC CGG CGT	MGN
Lys	K	AAA AAG	AAR
Met	M	ATG	ATG
Ile	I	ATA ATC ATT	ATH
Leu	L	CTA CTC CTG CTT TTA TTG	YTN
Val	V	GTA GTC GTG GTT	GTN
Phe	F	TTC TTT	TTY
Tyr	Y	TAC TAT	TAY
Trp	W	TGG	TGG
Ter	.	TAA TAG TGA	TRR
Asn|Asp	B		RAY
Glu|Gln	Z		SAR
Any	X		NNN

One of ordinary skill in the art will appreciate that some ambiguity is introduced in determining a degenerate codon, representative of all possible codons encoding each amino acid. For example, the degenerate codon for serine (WSN) can, in some circumstances, encode arginine (AGR), and the degenerate codon for arginine (MGN) can, in some circumstances, encode serine (AGY). A similar relationship exists between codons encoding phenylalanine and leucine. Thus, some polynucleotides encompassed by the degenerate sequence may encode variant amino acid sequences, but one of ordinary skill in the art can easily identify such variant sequences by reference to the amino acid sequence of SEQ ID NO:2 or SEQ ID NO:35. Variant sequences can be readily tested for functionality as described herein.

One of ordinary skill in the art will also appreciate that different species can exhibit "preferential codon usage." In general, see, Grantham, et al., Nuc. Acids Res. 8:1893-912, 1980; Haas, et al. Curr. Biol. 6:315-24, 1996; Wain-Hobson, et al., Gene 13:355-64, 1981; Grosjean and Fiers, Gene 18:199-209, 1982; Holm, Nuc. Acids Res. 14:3075-87, 1986; Ikemura, J. Mol. Biol. 158:573-97, 1982. As used herein, the term "preferential codon usage" or "preferential codons" is a term of art referring to protein translation codons that are most frequently used in cells of a certain species, thus favoring one or a few representatives of the possible codons encoding each amino acid (See Table 2). For example, the amino acid Threonine (Thr) may be encoded by ACA, ACC, ACG, or ACT, but in mammalian cells ACC is the most commonly used codon; in other species, for example, insect cells, yeast, viruses or bacteria, different Thr codons may be preferential. Preferential codons for a particular species can be introduced into the polynucleotides of the present invention by a variety of methods known in the art. Introduction of preferential codon sequences into recombinant DNA can, for example, enhance production of the protein by making protein translation more efficient within a particular cell type or species. Therefore, the degenerate codon sequences disclosed in SEQ ID NO:4 and SEQ ID NO:39 serve as a template for optimizing expression of polynucleotides in various cell types and species commonly used in the art and disclosed herein. Sequences containing preferential codons can be tested and optimized for expression in various species, and tested for functionality as disclosed herein.

Within preferred embodiments of the invention the isolated polynucleotides will hybridize to similar sized regions of SEQ ID NO:1, SEQ ID NO:34, or a sequence complementary thereto, under stringent conditions. In general, stringent conditions are selected to be about 5°C lower than the thermal melting point (T_m) for the specific sequence at a defined ionic strength and pH. The T_m is the temperature (under defined ionic strength and pH) at which 50% of the target sequence hybridizes to a perfectly matched probe. Numerous equations for calculating T_m are known in the art, and are specific for DNA, RNA and DNA-RNA hybrids and polynucleotide probe sequences of varying length (see, for example, Sambrook *et al.*, *Molecular Cloning: A Laboratory Manual*, Second Edition (Cold Spring Harbor Press 1989); Ausubel *et al.*, (eds.), *Current Protocols in Molecular Biology* (John Wiley and Sons, Inc. 1987); Berger and Kimmel (eds.), *Guide to Molecular Cloning Techniques*, (Academic Press, Inc. 1987); and Wetmur, *Crit. Rev. Biochem. Mol. Biol. 26*:227 (1990)). Sequence analysis software such as OLIGO 6.0 (LSR; Long Lake, MN) and *Primer Premier 4.0* (Premier Biosoft International; Palo Alto, CA), as well as sites on the Internet, are available tools for analyzing a given sequence and calculating T_m based on user defined criteria. Such programs can also analyze a given sequence under defined conditions and identify suitable probe sequences. Typically, hybridization of longer polynucleotide sequences, >50 base pairs, is performed at temperatures of about 20-25°C below the calculated T_m. For smaller probes, <50 base pairs, hybridization is typically carried out at the T_m or 5-10°C below. This allows for the maximum rate of hybridization for DNA-DNA and DNA-RNA hybrids. Higher degrees of stringency at lower temperatures can be achieved with the addition of formamide which reduces the T_m of the hybrid about 1°C for each 1% formamide in the buffer solution. Suitable stringent hybridization conditions are equivalent to about a 5 h to overnight incubation at about 42°C in a solution comprising: about 40-50% formamide, up to about 6X SSC, about 5X Denhardt's solution, zero up to about 10% dextran sulfate, and about 10-20 µg/ml denatured commercially-available carrier DNA. Generally, such stringent conditions include temperatures of 20-70°C and a hybridization buffer containing up to 6x SSC and 0-50% formamide; hybridization is then followed by washing filters in up to about 2X SSC. For example, a suitable wash stringency is equivalent to 0.1X SSC to

2X SSC, 0.1% SDS, at 55°C to 65°C. Different degrees of stringency can be used during hybridization and washing to achieve maximum specific binding to the target sequence. Typically, the washes following hybridization are performed at increasing degrees of stringency to remove non-hybridized polynucleotide probes from hybridized complexes. Stringent hybridization and wash conditions depend on the length of the probe, reflected in the Tm, hybridization and wash solutions used, and are routinely determined empirically by one of skill in the art.

As previously noted, the isolated polynucleotides of the present invention include DNA and RNA. Methods for preparing DNA and RNA are well known in the art. In general, RNA is isolated from a tissue or cell that produces large amounts of mouse zcytor10 RNA. Such tissues and cells are identified by Northern blotting (Thomas, Proc. Natl. Acad. Sci. USA 77:5201, 1980), and include PBLs, spleen, thymus, and lymph tissues, Raji cells, human erythroleukemia cell lines (e.g., TF-1), acute monocytic leukemia cell lines, other lymphoid and hematopoietic cell lines, and the like. Total RNA can be prepared using guanidinium isothiocyanate extraction followed by isolation by centrifugation in a CsCl gradient (Chirgwin et al., Biochemistry 18:52-94, 1979). Poly $(A)^+$ RNA is prepared from total RNA using the method of Aviv and Leder (Proc. Natl. Acad. Sci. USA 69:1408-12, 1972). Complementary DNA (cDNA) is prepared from poly$(A)^+$ RNA using known methods. In the alternative, genomic DNA can be isolated. Polynucleotides encoding mouse zcytor10 polypeptides are then identified and isolated by, for example, hybridization or polymerase chain reaction (PCR) (Mullis, U.S. Patent No. 4,683,202).

A full-length clone encoding mouse zcytor10 can be obtained by conventional cloning procedures. Complementary DNA (cDNA) clones are preferred, although for some applications (e.g., expression in transgenic animals) it may be preferable to use a genomic clone, or to modify a cDNA clone to include at least one genomic intron. Methods for preparing cDNA and genomic clones are well known and within the level of ordinary skill in the art, and include the use of the sequence disclosed herein, or parts thereof, for probing or priming a library. Expression libraries can be probed with antibodies to mouse zcytor10, receptor fragments, or other specific binding partners.

The polynucleotides of the present invention can also be synthesized using DNA synthesis machines. If chemically synthesized double stranded DNA is required for an application such as the synthesis of a DNA or a DNA fragment, then each complementary strand is made separately, for example via the phosphoramidite method known in the art. The production of short polynucleotides (60 to 80 bp) is technically straightforward and can be accomplished by synthesizing the complementary strands and then annealing them. However, for producing longer polynucleotides (longer than about 300 bp), special strategies are usually employed. For example, synthetic DNAs (double-stranded) are assembled in modular form from single-stranded fragments that are from 20 to 100 nucleotides in length. One method for building a synthetic DNA involves producing a set of overlapping, complementary oligonucleotides. Each internal section of the DNA has complementary 3' and 5' terminal extensions designed to base pair precisely with an adjacent section. After the DNA is assembled, the process is completed by ligating the nicks along the backbones of the two strands. In addition to the protein coding sequence, synthetic DNAs can be designed with terminal sequences that facilitate insertion into a restriction endonuclease site of a cloning vector. Alternative ways to prepare a full-length DNA are also known in the art. See Glick and Pasternak, Molecular Biotechnology, Principles & Applications of Recombinant DNA, (ASM Press, Washington, D.C. 1994); Itakura et al., Annu. Rev. Biochem. 53: 323-56, 1984 and Climie et al., Proc. Natl. Acad. Sci. USA 87:633-7, 1990.

The present invention further provides counterpart polypeptides and polynucleotides from other species (orthologs). These species include, but are not limited to mammalian, avian, amphibian, reptile, fish, insect and other vertebrate and invertebrate species. Of particular interest are zcytor10 polypeptides from other mammalian species, including human, other murine (e.g., rat), porcine, ovine, bovine, canine, feline, equine, and other primate polypeptides. Orthologs of mouse zcytor10 can be cloned using information and compositions provided by the present invention in combination with conventional cloning techniques. For example, a cDNA can be cloned using mRNA obtained from a human tissue or cell type that expresses zcytor10 as disclosed herein. Suitable sources of mRNA can be identified by probing Northern

blots with probes designed from the sequences disclosed herein. A library is then prepared from mRNA of a positive tissue or cell line. An orthologous zcytor10-encoding cDNA can then be isolated by a variety of methods, such as by probing with a complete or partial mouse cDNA or with one or more sets of degenerate probes based on the disclosed sequences. A cDNA can also be cloned using PCR (Mullis, supra.), using primers designed from the representative mouse zcytor10 sequence disclosed herein. Within an additional method, the cDNA library can be used to transform or transfect host cells, and expression of the cDNA of interest can be detected with an antibody to mouse zcytor10 polypeptide. Similar techniques can also be applied to the isolation of genomic clones.

A polynucleotide sequence for the rat ortholog of mouse zcytor10 receptor has been identified and is shown in SEQ ID NO:15 and the corresponding amino acid sequence shown in SEQ ID NO:16. Analysis of the rat zcytor10 polypeptide encoded by the DNA sequence of SEQ ID NO:15 revealed a partial sequence encoding 110 amino acids (SEQ ID NO:16) comprising the rat intracellular cytokine signaling domain including part of the transmembrane domain transmembrane domain (residues 1 (Ala) to 12 (Leu) of SEQ ID NO:16); a functional intracellular signaling domain (residues 13 (Arg) to 113 (Leu) of SEQ ID NO:16) which contains a "Box I" signaling site (residues 21 21 (Leu) to 28 (Pro) of SEQ ID NO:16), and a "Box II' signaling site (residues 59 (Glu) to 63 (Asp) of SEQ ID NO:16). A comparison of the rat and mouse amino acid sequences reveals that both the orthologous polypeptides contain corresponding structural features described above. The complete rat sequence can be obtained by performing routine 5' RACE using primers within SEQ ID NO:15. The corresponding polynucleotides encoding the rat zcytor10 polypeptide regions, domains, motifs, residues and sequences described above are as shown in SEQ ID NO:15.

Cytokine receptor subunits are characterized by a multi-domain structure comprising an extracellular domain, a transmembrane domain that anchors the polypeptide in the cell membrane, and an intracellular domain. The extracellular domain may be a ligand-binding domain, and the intracellular domain may be an effector domain involved in signal transduction, although ligand-binding and effector

functions may reside on separate subunits of a multimeric receptor. The ligand-binding domain may itself be a multi-domain structure. Multimeric receptors include homodimers (e.g., PDGF receptor αα and ββ isoforms, erythropoietin receptor, MPL, and G-CSF receptor), heterodimers whose subunits each have ligand-binding and effector domains (e.g., PDGF receptor αβ isoform), and multimers having component subunits with disparate functions (e.g., IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, and GM-CSF receptors). Some receptor subunits are common to a plurality of receptors. For example, the AIC2B subunit, which cannot bind ligand on its own but includes an intracellular signal transduction domain, is a component of IL-3, GM-CSF, and IL-5 receptors. Many cytokine receptors can be placed into one of four related families on the basis of the structure and function. Hematopoietic receptors, for example, are characterized by the presence of a domain containing conserved cysteine residues and the WSXWS motif (SEQ ID NO:3). Cytokine receptor structure has been reviewed by Urdal, Ann. Reports Med. Chem. 26:221-228, 1991 and Cosman, Cytokine 5:95-106, 1993. Under selective pressure for organisms to acquire new biological functions, new receptor family members likely arise from duplication of existing receptor genes leading to the existence of multi-gene families. Family members thus contain vestiges of the ancestral gene, and these characteristic features can be exploited in the isolation and identification of additional family members. Thus, the cytokine receptor superfamily is subdivided into several families, for example, the immunoglobulin family (including CSF-1, MGF, IL-1, and PDGF receptors); the hematopoietin family (including IL-2 receptor β-subunit, GM-CSF receptor α-subunit, GM-CSF receptor β-subunit; and G-CSF, EPO, IL-3, IL-4, IL-5, IL-6, IL-7, and IL-9 receptors); TNF receptor family (including TNF (p80) TNF (p60) receptors, CD27, CD30, CD40, Fas, and NGF receptor).

Analysis of the mouse zcytor10 sequence suggests that it is a member of the same receptor subfamily as the EPO and growth hormone receptors. Certain receptors in this subfamily (e.g., G-CSF) associate to form homodimers that transduce a signal. Other members of the subfamily (e.g., IL-6, IL-11, and LIF receptors) combine with a second subunit (termed a β-subunit) to bind ligand and transduce a signal. Specific β-subunits associate with a plurality of specific cytokine receptor subunits.

For example, the β-subunit gp130 (Hibi et al., Cell 63:1149-1157, 1990) associates with receptor subunits specific for IL-6, IL-11, and LIF (Gearing et al., EMBO J. 10:2839-2848, 1991; Gearing et al., U.S. Patent No. 5,284,755). Oncostatin M binds to a heterodimer of LIF receptor and gp130. CNTF binds to trimeric receptors comprising CNTF receptor, LIF receptor, and gp130 subunits.

Zcytor10 shows sequence and structural homology to IL-2Rγ (gamma common receptor; γc), IL-3R discussed above, and IL-7R that are known to form heterodimeric or multimeric complexes with other cytokine receptor subunits. For example, IL-7Rα heterodimerizes with gamma common to form the receptor for the IL-7 ligand. Moreover, another heterodimeric receptor, called TSLP-R, has also been shown to heterodimerize with IL-7Rα to form the receptor for a novel ligand, TSLP (Levine, SD et al., J. Immunol. 162:677-683, 1999; Isaksen, DE et al., J. Immunol. 163:5971-5977, 1999; Ray, RJ et al., Eur. J. Immunol. 26:10-16, 1996; Friend, SL et al., Exper. Hematol. 22:321-328, 1994). It is therefore possible that zcytor10 forms heterodimerizes or forms multimers with other receptor subunits in the gamma common receptor family, creating receptors for other novel cytokines. These cytokines may have functions that overlap those of the gamma common-interacting cytokine, as is the case with IL7 and TSLP. However, it is also possible that the effects may be quite divergent, or present at different times or under different conditions. Therefore it is important to identify cytokines that interact with zcytor10 in combination with other cytokine receptor subunits.

An assay cell line can be created by transfection of zcytor10 and an additional cytokine receptor subunit into a cell line such as BaF3, described herein. Known cytokines and a collection of conditioned media from at least 100 cell lines, as well as tissue preparations, and purified cytokine preparations can be rapidly tested for the ability to support proliferation of this co-transfected cell line. A sample that contains such an activity is further evaluated in the presence of neutralizing antibodies against gamma common receptor (e.g., anti-IL-2 receptor monoclonal antibodies from PharMingen International, San Diego, CA) to confirm that the endogenous gamma common in the BaF3 cells is not participating in the receptor complex. Moreover

specificity can be evaluated by showing inhibition of proliferation by antibodies to the co-transfected subunit (commercially available from various manufacturer's), anti-zcytor10 antibodies described herein, or soluble zcytor10 receptors described herein. A cell line, which produces an activity that supports non-gamma common-mediated proliferation, can then be used to produce a cDNA library for ligand cloning. Such Baf3 assay cell lines can be created with zcytor10 co-expressed with other receptor complexes including but not limited to zcytor10 receptor in combination with an cytokine receptor fusion comprising one or more of the IL-2 receptor components (IL-2Rα, IL-2Rβ, IL-2Rγ), zcytor10 receptor with one or more of the IL-4/IL-13 receptor family receptor components (IL-4Rα, IL-13Rα, IL-13Rα'), as well as other Interleukin receptors (e.g., IL-15 Rα, IL-7Rα, IL-9Rα, IL-21R (Zalpha11 receptor; commonly owned US Pat. Application No. 09/404,641).

Those skilled in the art will recognize that the sequence disclosed in SEQ ID NO:1 represents a single allele of mouse zcytor10 and that allelic variation and alternative splicing are expected to occur. Allelic variants of this sequence can be cloned by probing cDNA or genomic libraries from different individuals according to standard procedures. Allelic variants of the DNA sequence shown in SEQ ID NO:1, including those containing silent mutations and those in which mutations result in amino acid sequence changes, are within the scope of the present invention, as are proteins which are allelic variants of SEQ ID NO:2. cDNAs generated from alternatively spliced mRNAs, such as SEQ ID NO:34, which retain the properties of the mouse zcytor10 polypeptide are included within the scope of the present invention, as are polypeptides encoded by such cDNAs and mRNAs. Allelic variants and splice variants of these sequences can be cloned by probing cDNA or genomic libraries from different individuals or tissues according to standard procedures known in the art.

The present invention also provides isolated mouse zcytor10 polypeptides that are substantially similar to the polypeptides of SEQ ID NO:2, or SEQ ID NO:35, and their orthologs. The term "substantially similar" is used herein to denote polypeptides having at least 70%, more preferably at least 80%, sequence identity to the sequences shown in SEQ ID NO:2, or SEQ ID NO:35 or their orthologs. Such polypeptides will more preferably be at least 90% identical, and most preferably 95% or

more identical to SEQ ID NO:2 or SEQ ID NO:35 or its orthologs.) Percent sequence identity is determined by conventional methods. See, for example, Altschul et al., Bull. Math. Bio. 48: 603-616, 1986 and Henikoff and Henikoff, Proc. Natl. Acad. Sci. USA 89:10915-10919, 1992. Briefly, two amino acid sequences are aligned to optimize the alignment scores using a gap opening penalty of 10, a gap extension penalty of 1, and the "blosum 62" scoring matrix of Henikoff and Henikoff (ibid.) as shown in Table 3 (amino acids are indicated by the standard one-letter codes). The percent identity is then calculated as:

$$\frac{\text{Total number of identical matches}}{\text{[length of the longer sequence plus the number of gaps introduced into the longer sequence in order to align the two sequences]}} \times 100$$

Table 3

	A	R	N	D	C	Q	E	G	H	I	L	K	M	F	P	S	T	W	Y	V
A	4																			
R	-1	5																		
N	-2	0	6																	
D	-2	-2	1	6																
C	0	-3	-3	-3	9															
Q	-1	1	0	0	-3	5														
E	-1	0	0	2	-4	2	5													
G	0	-2	0	-1	-3	-2	-2	6												
H	-2	0	1	-1	-3	0	0	-2	8											
I	-1	-3	-3	-3	-1	-3	-3	-4	-3	4										
L	-1	-2	-3	-4	-1	-2	-3	-4	-3	2	4									
K	-1	2	0	-1	-3	1	1	-2	-1	-3	-2	5								
M	-1	-1	-2	-3	-1	0	-2	-3	-2	1	2	-1	5							
F	-2	-3	-3	-3	-2	-3	-3	-3	-1	0	0	-3	0	6						
P	-1	-2	-2	-1	-3	-1	-1	-2	-2	-3	-3	-1	-2	-4	7					
S	1	-1	1	0	-1	0	0	0	-1	-2	-2	0	-1	-2	-1	4				
T	0	-1	0	-1	-1	-1	-1	-2	-2	-1	-1	-1	-1	-2	-1	1	5			
W	-3	-3	-4	-4	-2	-2	-3	-2	-2	-3	-2	-3	-1	1	-4	-3	-2	11		
Y	-2	-2	-2	-3	-2	-1	-2	-3	2	-1	-1	-2	-1	3	-3	-2	-2	2	7	
V	0	-3	-3	-3	-1	-2	-2	-3	-3	3	1	-2	1	-1	-2	-2	0	-3	-1	4

Sequence identity of polynucleotide molecules is determined by similar methods using a ratio as disclosed above.

Those skilled in the art appreciate that there are many established algorithms available to align two amino acid sequences. The "FASTA" similarity search algorithm of Pearson and Lipman is a suitable protein alignment method for examining the level of identity shared by an amino acid sequence disclosed herein and the amino acid sequence of a putative variant zpep14. The FASTA algorithm is described by Pearson and Lipman, *Proc. Nat'l Acad. Sci. USA 85*:2444 (1988), and by Pearson, *Meth. Enzymol. 183*:63 (1990).

Briefly, FASTA first characterizes sequence similarity by identifying regions shared by the query sequence (*e.g.*, SEQ ID NO:2) and a test sequence that have either the highest density of identities (if the ktup variable is 1) or pairs of identities (if ktup=2), without considering conservative amino acid substitutions, insertions, or deletions. The ten regions with the highest density of identities are then rescored by comparing the similarity of all paired amino acids using an amino acid substitution matrix, and the ends of the regions are "trimmed" to include only those residues that contribute to the highest score. If there are several regions with scores greater than the "cutoff" value (calculated by a predetermined formula based upon the length of the sequence and the ktup value), then the trimmed initial regions are examined to determine whether the regions can be joined to form an approximate alignment with gaps. Finally, the highest scoring regions of the two amino acid sequences are aligned using a modification of the Needleman-Wunsch-Sellers algorithm (Needleman and Wunsch, *J. Mol. Biol. 48*:444 (1970); Sellers, *SIAM J. Appl. Math. 26*:787 (1974)), which allows for amino acid insertions and deletions. Preferred parameters for FASTA analysis are: ktup=1, gap opening penalty=10, gap extension penalty=1, and substitution matrix=BLOSUM62. These parameters can be introduced into a FASTA program by modifying the scoring matrix file ("SMATRIX"), as explained in Appendix 2 of Pearson, *Meth. Enzymol. 183*:63 (1990).

FASTA can also be used to determine the sequence identity of nucleic acid molecules using a ratio as disclosed above. For nucleotide sequence comparisons,

the ktup value can range between one to six, preferably from three to six, most preferably three, with other FASTA program parameters set as default.

The BLOSUM62 table (Table 3) is an amino acid substitution matrix derived from about 2,000 local multiple alignments of protein sequence segments, representing highly conserved regions of more than 500 groups of related proteins (Henikoff and Henikoff, *Proc. Nat'l Acad. Sci. USA 89*:10915 (1992)). Accordingly, the BLOSUM62 substitution frequencies can be used to define conservative amino acid substitutions that may be introduced into the amino acid sequences of the present invention. Although it is possible to design amino acid substitutions based solely upon chemical properties (as discussed below), the language "conservative amino acid substitution" preferably refers to a substitution represented by a BLOSUM62 value of greater than -1. For example, an amino acid substitution is conservative if the substitution is characterized by a BLOSUM62 value of 0, 1, 2, or 3. According to this system, preferred conservative amino acid substitutions are characterized by a BLOSUM62 value of at least 1 (e.g., 1, 2 or 3), while more preferred conservative amino acid substitutions are characterized by a BLOSUM62 value of at least 2 (e.g., 2 or 3).

Variant or substantially homologous mouse zcytor10 polypeptides are characterized as having one or more amino acid substitutions, deletions or additions. These changes are preferably of a minor nature, that is conservative amino acid substitutions (see Table 4) and other substitutions that do not significantly affect the folding or activity of the polypeptide; small deletions, typically of one to about 30 amino acids; and small amino- or carboxyl-terminal extensions, such as an amino-terminal methionine residue, a small linker peptide of up to about 20-25 residues, or an affinity tag. The present invention thus includes polypeptides of from about 489 to about 568 amino acid residues that comprise a sequence that is at least 80%, preferably at least 90%, and more preferably 95% or more identical to the corresponding region of SEQ ID NO:2 or SEQ ID NO:35. Polypeptides comprising affinity tags can further comprise a proteolytic cleavage site between the mouse zcytor10 polypeptide and the affinity tag. Suitable sites include thrombin cleavage sites and factor Xa cleavage sites.

Table 4
Conservative amino acid substitutions

Basic:	arginine
	lysine
	histidine
Acidic:	glutamic acid
	aspartic acid
Polar:	glutamine
	asparagine

Table 4 cont.

Hydrophobic:	leucine
	isoleucine
	valine
Aromatic:	phenylalanine
	tryptophan
	tyrosine
Small:	glycine
	alanine
	serine
	threonine
	methionine

The present invention further provides a variety of other polypeptide fusions and related multimeric proteins comprising one or more polypeptide fusions. For example, a mouse zcytor10 polypeptide can be prepared as a fusion to a dimerizing protein as disclosed in U.S. Patents Nos. 5,155,027 and 5,567,584. Preferred dimerizing proteins in this regard include immunoglobulin constant region domains. Immunoglobulin-zcytor10 polypeptide fusions can be expressed in genetically engineered cells to produce a variety of multimeric mouse zcytor10 analogs. Auxiliary domains can be fused to mouse zcytor10 polypeptides to target them to specific cells, tissues, or macromolecules (e.g., collagen). A mouse zcytor10 polypeptide can be fused

to two or more moieties, such as an affinity tag for purification and a targeting domain. Polypeptide fusions can also comprise one or more cleavage sites, particularly between domains. See, Tuan et al., Connective Tissue Research 34:1-9, 1996.

The proteins of the present invention can also comprise non-naturally occurring amino acid residues. Non-naturally occurring amino acids include, without limitation, *trans*-3-methylproline, 2,4-methanoproline, *cis*-4-hydroxyproline, *trans*-4-hydroxyproline, *N*-methylglycine, *allo*-threonine, methylthreonine, hydroxyethylcysteine, hydroxyethylhomocysteine, nitroglutamine, homoglutamine, pipecolic acid, thiazolidine carboxylic acid, dehydroproline, 3- and 4-methylproline, 3,3-dimethylproline, *tert*-leucine, norvaline, 2-azaphenylalanine, 3-azaphenylalanine, 4-azaphenylalanine, and 4-fluorophenylalanine. Several methods are known in the art for incorporating non-naturally occurring amino acid residues into proteins. For example, an *in vitro* system can be employed wherein nonsense mutations are suppressed using chemically aminoacylated suppressor tRNAs. Methods for synthesizing amino acids and aminoacylating tRNA are known in the art. Transcription and translation of plasmids containing nonsense mutations is carried out in a cell-free system comprising an *E. coli* S30 extract and commercially available enzymes and other reagents. Proteins are purified by chromatography. See, for example, Robertson et al., J. Am. Chem. Soc. 113:2722, 1991; Ellman et al., Methods Enzymol. 202:301, 1991; Chung et al., Science 259:806-9, 1993; and Chung et al., Proc. Natl. Acad. Sci. USA 90:10145-9, 1993). In a second method, translation is carried out in *Xenopus* oocytes by microinjection of mutated mRNA and chemically aminoacylated suppressor tRNAs (Turcatti et al., J. Biol. Chem. 271:19991-8, 1996). Within a third method, *E. coli* cells are cultured in the absence of a natural amino acid that is to be replaced (e.g., phenylalanine) and in the presence of the desired non-naturally occurring amino acid(s) (e.g., 2-azaphenylalanine, 3-azaphenylalanine, 4-azaphenylalanine, or 4-fluorophenylalanine). The non-naturally occurring amino acid is incorporated into the protein in place of its natural counterpart. See, Koide et al., Biochem. 33:7470-7476, 1994. Naturally occurring amino acid residues can be converted to non-naturally occurring species by *in vitro* chemical modification. Chemical modification can be combined with site-directed mutagenesis

to further expand the range of substitutions (Wynn and Richards, Protein Sci. 2:395-403, 1993).

A limited number of non-conservative amino acids, amino acids that are not encoded by the genetic code, non-naturally occurring amino acids, and unnatural amino acids may be substituted for mouse zcytor10 amino acid residues.

Essential amino acids in the polypeptides of the present invention can be identified according to procedures known in the art, such as site-directed mutagenesis or alanine-scanning mutagenesis (Cunningham and Wells, Science 244: 1081-5, 1989; Bass et al., Proc. Natl. Acad. Sci. USA 88:4498-502, 1991). In the latter technique, single alanine mutations are introduced at every residue in the molecule, and the resultant mutant molecules are tested for biological activity (e.g. ligand binding and signal transduction) as disclosed below to identify amino acid residues that are critical to the activity of the molecule. See also, Hilton et al., J. Biol. Chem. 271:4699-4708, 1996. Sites of ligand-receptor, protein-protein or other biological interaction can also be determined by physical analysis of structure, as determined by such techniques as nuclear magnetic resonance, crystallography, electron diffraction or photoaffinity labeling, in conjunction with mutation of putative contact site amino acids. See, for example, de Vos et al., Science 255:306-312, 1992; Smith et al., J. Mol. Biol. 224:899-904, 1992; Wlodaver et al., FEBS Lett. 309:59-64, 1992. The identities of essential amino acids can also be inferred from analysis of homologies with related receptors.

Determination of amino acid residues that are within regions or domains that are critical to maintaining structural integrity can be determined. Within these regions one can determine specific residues that will be more or less tolerant of change and maintain the overall tertiary structure of the molecule. Methods for analyzing sequence structure include, but are not limited to, alignment of multiple sequences with high amino acid or nucleotide identity and computer analysis using available software (e.g., the Insight II® viewer and homology modeling tools; MSI, San Diego, CA), secondary structure propensities, binary patterns, complementary packing and buried polar interactions (Barton, Current Opin. Struct. Biol. 5:372-376, 1995 and Cordes et al., Current Opin. Struct. Biol. 6:3-10, 1996). In general, when designing modifications

to molecules or identifying specific fragments determination of structure will be accompanied by evaluating activity of modified molecules.

Amino acid sequence changes are made in zcytor10 polypeptides, including zcytor10 soluble receptors and heterodimeric receptor polypeptides, so as to minimize disruption of higher order structure essential to biological activity. For example, when the zcytor10 polypeptides, zcytor10 soluble receptors and heterodimeric receptor polypeptides comprise one or more helices, changes in amino acid residues will be made so as not to disrupt the helix geometry and other components of the molecule where changes in conformation abate some critical function, for example, binding of the molecule to its binding partners. The effects of amino acid sequence changes can be predicted by, for example, computer modeling as disclosed above or determined by analysis of crystal structure (see, e.g., Lapthorn et al., Nat. Struct. Biol. 2:266-268, 1995). Other techniques that are well known in the art compare folding of a variant protein to a standard molecule (e.g., the native protein). For example, comparison of the cysteine pattern in a variant and standard molecules can be made. Mass spectrometry and chemical modification using reduction and alkylation provide methods for determining cysteine residues which are associated with disulfide bonds or are free of such associations (Bean et al., Anal. Biochem. 201:216-226, 1992; Gray, Protein Sci. 2:1732-1748, 1993; and Patterson et al., Anal. Chem. 66:3727-3732, 1994). It is generally believed that if a modified molecule does not have the same disulfide bonding pattern as the standard molecule folding would be affected. Another well known and accepted method for measuring folding is circular dichrosism (CD). Measuring and comparing the CD spectra generated by a modified molecule and standard molecule is routine (Johnson, Proteins 7:205-214, 1990). Crystallography is another well known method for analyzing folding and structure. Nuclear magnetic resonance (NMR), digestive peptide mapping and epitope mapping are also known methods for analyzing folding and structural similarities between proteins and polypeptides (Schaanan et al., Science 257:961-964, 1992).

A Hopp/Woods hydrophilicity profile of the zcytor10 polypeptides, zcytor10 soluble receptors and heterodimeric receptor protein sequence as shown in SEQ ID NO:2 and SEQ ID NO:35 can be generated (Hopp et al., Proc. Natl. Acad.

Sci.78:3824-3828, 1981; Hopp, J. Immun. Meth. 88:1-18, 1986 and Triquier et al., Protein Engineering 11:153-169, 1998). The profile is based on a sliding six-residue window. Buried G, S, and T residues and exposed H, Y, and W residues were ignored. For example, in zcytor10 polypeptide hydrophilic regions include (1) amino acid number 150 (Arg) to amino acid number 155 (Asp) of SEQ ID NO:2; (2) amino acid number 254 (Arg) to amino acid number 259 (Ala) of SEQ ID NO:2; (3) amino acid number 296 (Ala) to amino acid number 301 (Glu) of SEQ ID NO:2; (4) amino acid number 297 (Arg) to amino acid number 302 (Asp) of SEQ ID NO:2; and (5) amino acid number 310 (Lys) to amino acid number 315 (Glu) of SEQ ID NO:2. The corresponding zcytor10 hydrophilic peptides of SEQ ID NO:35 are also included with comparison of the above hydrophilic peptides SEQ ID NO:2 in reference to SEQ ID NO:35.

Those skilled in the art will recognize that hydrophilicity or hydrophobicity will be taken into account when designing modifications in the amino acid sequence of a zcytor10 polypeptides, zcytor10 soluble receptors and heterodimeric receptor polypeptides, so as not to disrupt the overall structural and biological profile. Of particular interest for replacement are hydrophobic residues selected from the group consisting of Val, Leu and Ile or the group consisting of Met, Gly, Ser, Ala, Tyr and Trp. For example, residues tolerant of substitution could include such as shown in SEQ ID NO: 2 and SEQ ID NO:35. Cysteine residues will be relatively intolerant of substitution.

The identities of essential amino acids can also be inferred from analysis of sequence similarity between class I cytokine receptor family members with zcytor10 polypeptides, including zcytor10 soluble receptors and heterodimeric receptors. Using methods such as "FASTA" analysis described previously, regions of high similarity are identified within a family of proteins and used to analyze amino acid sequence for conserved regions. An alternative approach to identifying a variant zcytor10, zcytor10 soluble receptors and heterodimeric receptor polynucleotides on the basis of structure is to determine whether a nucleic acid molecule encoding a potential variant polynucleotide can hybridize to a nucleic acid molecule having the nucleotide sequence of SEQ ID NO:1, or SEQ ID NO:34 as discussed above.

Other methods of identifying essential amino acids in the polypeptides of the present invention are procedures known in the art, such as site-directed mutagenesis or alanine-scanning mutagenesis (Cunningham and Wells, Science 244:1081 (1989), Bass et al., Proc. Natl Acad. Sci. USA 88:4498 (1991), Coombs and Corey, "Site-Directed Mutagenesis and Protein Engineering," in Proteins: Analysis and Design, Angeletti (ed.), pages 259-311 (Academic Press, Inc. 1998)). In the latter technique, single alanine mutations are introduced at every residue in the molecule, and the resultant mutant molecules are tested for biological activity as disclosed below to identify amino acid residues that are critical to the activity of the molecule. See also, Hilton *et al., J. Biol. Chem. 271*:4699 (1996).

The present invention also includes functional fragments of zcytor10 polypeptides, zcytor10 soluble receptors and heterodimeric receptor polypeptides and nucleic acid molecules encoding such functional fragments. A "functional" zcytor10 polypeptide, includes zcytor10 soluble receptors and heterodimeric receptors or fragment thereof defined herein is characterized by its proliferative or differentiating activity, by its ability to induce or inhibit specialized cell functions, or by its ability to bind specifically to a either soluble or immobilized anti- zcytor10 antibody, a zcytor10 ligand or cytokine receptor subunit. As previously described herein, the zcytor10 receptor is characterized by a class I cytokine receptor structure. Thus, the present invention further provides fusion proteins encompassing: (a) homodimeric or multimeric polypeptide molecules comprising an extracellular or intracellular domain described herein; and (b) functional fragments comprising one or more of these domains. The other polypeptide portion of the fusion protein may be contributed by another class I cytokine receptor, for example, IL-2Rγ, IL-2 receptor β-subunit and the β-common receptor (i.e., IL3, IL-5, and GM-CSF receptor β-subunits), IL-13α, IL-13α', IL-7α, IL-15, or IL-21 (zalpha11) receptor subunits, or by a non-native and/or an unrelated secretory signal peptide that facilitates secretion of the soluble fusion protein.

Routine deletion analyses of nucleic acid molecules can be performed to obtain functional fragments of a nucleic acid molecule that encode zcytor10 polypeptides, zcytor10 soluble receptors and heterodimeric receptor polypeptides. As an illustration, DNA molecules having the nucleotide sequence of SEQ ID NO:1 or

fragments thereof, can be digested with *Bal*31 nuclease to obtain a series of nested deletions. These DNA fragments are then inserted into expression vectors in proper reading frame, and the expressed polypeptides are isolated and tested for zcytor10 polypeptides, including zcytor10 soluble receptors and heterodimeric receptor activity, or for the ability to bind anti-zcytor10 antibodies or zcytor10 receptor. One alternative to exonuclease digestion is to use oligonucleotide-directed mutagenesis to introduce deletions or stop codons to specify production of a desired zcytor10 polypeptides, including zcytor10 soluble receptors and heterodimeric receptor fragment. Alternatively, particular fragments of zcytor10 polypeptides, including zcytor10 soluble receptors and heterodimeric receptor polynucleotides can be synthesized using the polymerase chain reaction.

Standard methods for identifying functional domains are well-known to those of skill in the art. For example, studies on the truncation at either or both termini of interferons have been summarized by Horisberger and Di Marco, Pharmac. Ther. 66:507 (1995). Moreover, standard techniques for functional analysis of proteins are described by, for example, Treuter et al., Molec. Gen. Genet. 240:113 (1993); Content et al., "Expression and preliminary deletion analysis of the 42 kDa 2-5A synthetase induced by human interferon," in Biological Interferon Systems, Proceedings of ISIR-TNO Meeting on Interferon Systems, Cantell (ed.), pages 65-72 (Nijhoff 1987); Herschman, "The EGF Receptor," in Control of Animal Cell Proliferation 1, Boynton et al., (eds.) pages 169-199 (Academic Press 1985); Coumailleau et al., J. Biol. Chem. 270:29270 (1995); Fukunaga et al., J. Biol. Chem. 270:25291 (1995); Yamaguchi et al., Biochem. Pharmacol. 50:1295 (1995); and Meisel et al., Plant Molec. Biol. 30:1 (1996).

Multiple amino acid substitutions can be made and tested using known methods of mutagenesis and screening, such as those disclosed by Reidhaar-Olson and Sauer (Science 241:53-57, 1988) or Bowie and Sauer (Proc. Natl. Acad. Sci. USA 86:2152-2156, 1989). Briefly, these authors disclose methods for simultaneously randomizing two or more positions in a polypeptide, selecting for functional polypeptide, and then sequencing the mutagenized polypeptides to determine the spectrum of allowable substitutions at each position. Other methods that can be used include phage display (e.g., Lowman et al., Biochem. 30:10832-10837, 1991; Ladner et

al., U.S. Patent No. 5,223,409; Huse, WIPO Publication WO 92/062045) and region-directed mutagenesis (Derbyshire et al., Gene 46:145, 1986; Ner et al., DNA 7:127, 1988).

Variants of the disclosed mouse zcytor10 DNA and polypeptide sequences can be generated through DNA shuffling as disclosed by Stemmer, Nature 370:389-91, 1994, Stemmer, Proc. Natl. Acad. Sci. USA 91:10747-51, 1994 and WIPO Publication WO 97/20078. Briefly, variant DNAs are generated by *in vitro* homologous recombination by random fragmentation of a parent DNA followed by reassembly using PCR, resulting in randomly introduced point mutations. This technique can be modified by using a family of parent DNAs, such as allelic variants or DNAs from different species, to introduce additional variability into the process. Selection or screening for the desired activity, followed by additional iterations of mutagenesis and assay provides for rapid "evolution" of sequences by selecting for desirable mutations while simultaneously selecting against detrimental changes.

Mutagenesis methods as disclosed herein can be combined with high-throughput, automated screening methods to detect activity of cloned, mutagenized mouse zcytor10 receptor polypeptides in host cells. Preferred assays in this regard include cell proliferation assays and biosensor-based ligand-binding assays, which are described below. Mutagenized DNA molecules that encode active receptors or portions thereof (e.g., ligand-binding fragments, signaling domains, and the like) can be recovered from the host cells and rapidly sequenced using modern equipment. These methods allow the rapid determination of the importance of individual amino acid residues in a polypeptide of interest, and can be applied to polypeptides of unknown structure.

Using the methods discussed herein, one of ordinary skill in the art can identify and/or prepare a variety of polypeptide fragments or variants of SEQ ID NO:2 or SEQ ID NO:35 that retain the signal transduction or ligand binding activity. For example, one can make a mouse zcytor10 "soluble receptor" by preparing a variety of polypeptides that are substantially homologous to the cytokine-binding domain (residues 15 (Cys) to 230 (Pro) of SEQ ID NO:2; residues 17 (Ala) to 232 (Pro) of SEQ ID NO:35) or allelic variants or species orthologs thereof) and retain ligand-binding

activity of the wild-type mouse zcytor10 protein. Such polypeptides may include additional amino acids from, for example, part or all of the transmembrane and intracellular domains. Such polypeptides may also include additional polypeptide segments as generally disclosed herein such as labels, affinity tags, and the like.

For any mouse zcytor10 polypeptide, including variants, soluble receptors, and fusion polypeptides or proteins, one of ordinary skill in the art can readily generate a fully degenerate polynucleotide sequence encoding that variant using the information set forth in Tables 1 and 2 above.

The mouse zcytor10 polypeptides of the present invention, including full-length polypeptides, biologically active fragments, and fusion polypeptides, can be produced in genetically engineered host cells according to conventional techniques. Suitable host cells are those cell types that can be transformed or transfected with exogenous DNA and grown in culture, and include bacteria, fungal cells, and cultured higher eukaryotic cells. Eukaryotic cells, particularly cultured cells of multicellular organisms, are preferred. Techniques for manipulating cloned DNA molecules and introducing exogenous DNA into a variety of host cells are disclosed by Sambrook et al., Molecular Cloning: A Laboratory Manual, 2nd ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989, and Ausubel et al., eds., Current Protocols in Molecular Biology, John Wiley and Sons, Inc., NY, 1987.

In general, a DNA sequence encoding a mouse zcytor10 polypeptide is operably linked to other genetic elements required for its expression, generally including a transcription promoter and terminator, within an expression vector. The vector will also commonly contain one or more selectable markers and one or more origins of replication, although those skilled in the art will recognize that within certain systems selectable markers may be provided on separate vectors, and replication of the exogenous DNA may be provided by integration into the host cell genome. Selection of promoters, terminators, selectable markers, vectors and other elements is a matter of routine design within the level of ordinary skill in the art. Many such elements are described in the literature and are available through commercial suppliers.

To direct a mouse zcytor10 polypeptide into the secretory pathway of a host cell, a secretory signal sequence (also known as a leader sequence, prepro sequence

or pre sequence) is provided in the expression vector. The secretory signal sequence may be that of mouse zcytor10, or may be derived from another secreted protein (e.g., t-PA) or synthesized *de novo*. The secretory signal sequence is operably linked to the mouse zcytor10 DNA sequence, i.e., the two sequences are joined in the correct reading frame and positioned to direct the newly synthesized polypeptide into the secretory pathway of the host cell. Secretory signal sequences are commonly positioned 5' to the DNA sequence encoding the polypeptide of interest, although certain secretory signal sequences may be positioned elsewhere in the DNA sequence of interest (see, e.g., Welch et al., U.S. Patent No. 5,037,743; Holland et al., U.S. Patent No. 5,143,830).

Alternatively, the secretory signal sequence contained in the polypeptides of the present invention is used to direct other polypeptides into the secretory pathway. The present invention provides for such fusion polypeptides. A signal fusion polypeptide can be made wherein a secretory signal sequence derived from amino acid 1 (Met) to amino acid 19 (Gly) of SEQ ID NO:2, or amino acid 1 (Met) to amino acid 16 (Ala) of SEQ ID NO:35, is operably linked to another polypeptide using methods known in the art and disclosed herein. The secretory signal sequence contained in the fusion polypeptides of the present invention is preferably fused amino-terminally to an additional peptide to direct the additional peptide into the secretory pathway. Such constructs have numerous applications known in the art. For example, these novel secretory signal sequence fusion constructs can direct the secretion of an active component of a normally non-secreted protein. Such fusions may be used *in vivo* or *in vitro* to direct peptides through the secretory pathway.

Cultured mammalian cells are suitable hosts within the present invention. Methods for introducing exogenous DNA into mammalian host cells include calcium phosphate-mediated transfection (Wigler et al., Cell 14:725, 1978; Corsaro and Pearson, Somatic Cell Genetics 7:603, 1981: Graham and Van der Eb, Virology 52:456, 1973), electroporation (Neumann et al., EMBO J. 1:841-845, 1982), DEAE-dextran mediated transfection (Ausubel et al., ibid.), and liposome-mediated transfection (Hawley-Nelson et al., Focus 15:73, 1993; Ciccarone et al., Focus 15:80, 1993, and viral vectors (Miller and Rosman, BioTechniques 7:980-90, 1989; Wang and Finer, Nature Med. 2:714-716, 1996). The production of recombinant polypeptides in

cultured mammalian cells is disclosed, for example, by Levinson et al., U.S. Patent No. 4,713,339; Hagen et al., U.S. Patent No. 4,784,950; Palmiter et al., U.S. Patent No. 4,579,821; and Ringold, U.S. Patent No. 4,656,134. Suitable cultured mammalian cells include the COS-1 (ATCC No. CRL 1650), COS-7 (ATCC No. CRL 1651), BHK (ATCC No. CRL 1632), BHK 570 (ATCC No. CRL 10314), 293 (ATCC No. CRL 1573; Graham et al., J. Gen. Virol. 36:59-72, 1977) and Chinese hamster ovary (e.g. CHO-K1; ATCC No. CCL 61) cell lines. Additional suitable cell lines are known in the art and available from public depositories such as the American Type Culture Collection, Rockville, Maryland. In general, strong transcription promoters are preferred, such as promoters from SV-40 or cytomegalovirus. See, e.g., U.S. Patent No. 4,956,288. Other suitable promoters include those from metallothionein genes (U.S. Patent Nos. 4,579,821 and 4,601,978) and the adenovirus major late promoter.

Drug selection is generally used to select for cultured mammalian cells into which foreign DNA has been inserted. Such cells are commonly referred to as "transfectants". Cells that have been cultured in the presence of the selective agent and are able to pass the gene of interest to their progeny are referred to as "stable transfectants." A preferred selectable marker is a gene encoding resistance to the antibiotic neomycin. Selection is carried out in the presence of a neomycin-type drug, such as G-418 or the like. Selection systems can also be used to increase the expression level of the gene of interest, a process referred to as "amplification." Amplification is carried out by culturing transfectants in the presence of a low level of the selective agent and then increasing the amount of selective agent to select for cells that produce high levels of the products of the introduced genes. A preferred amplifiable selectable marker is dihydrofolate reductase, which confers resistance to methotrexate. Other drug resistance genes (e.g. hygromycin resistance, multi-drug resistance, puromycin acetyltransferase) can also be used. Alternative markers that introduce an altered phenotype, such as green fluorescent protein, or cell surface proteins such as CD4, CD8, Class I MHC, placental alkaline phosphatase may be used to sort transfected cells from untransfected cells by such means as FACS sorting or magnetic bead separation technology.

Other higher eukaryotic cells can also be used as hosts, including plant cells, insect cells and avian cells. The use of *Agrobacterium rhizogenes* as a vector for expressing genes in plant cells has been reviewed by Sinkar et al., J. Biosci. (Bangalore) 11:47-58, 1987. Transformation of insect cells and production of foreign polypeptides therein is disclosed by Guarino et al., U.S. Patent No. 5,162,222 and WIPO publication WO 94/06463. Insect cells can be infected with recombinant baculovirus, commonly derived from *Autographa californica nuclear polyhedrosis virus* (AcNPV). See, King, L.A. and Possee, R.D., The Baculovirus Expression System: A Laboratory Guide, London, Chapman & Hall; O'Reilly, D.R. et al., Baculovirus Expression Vectors: A Laboratory Manual, New York, Oxford University Press., 1994; and, Richardson, C. D., Ed., Baculovirus Expression Protocols. Methods in Molecular Biology, Totowa, NJ, Humana Press, 1995. A second method of making recombinant mouse zcytor10 baculovirus utilizes a transposon-based system described by Luckow (Luckow, V.A, et al., J Virol 67:4566-79, 1993). This system, which utilizes transfer vectors, is sold in the Bac-to-Bac™ kit (Life Technologies, Rockville, MD). This system utilizes a transfer vector, pFastBac1™ (Life Technologies) containing a Tn7 transposon to move the DNA encoding the mouse zcytor10 polypeptide into a baculovirus genome maintained in E. coli as a large plasmid called a "bacmid." See, Hill-Perkins, M.S. and Possee, R.D., J Gen Virol 71:971-6, 1990; Bonning, B.C. et al., J Gen Virol 75:1551-6, 1994; and, Chazenbalk, G.D., and Rapoport, B., J Biol Chem 270:1543-9, 1995. In addition, transfer vectors can include an in-frame fusion with DNA encoding an epitope tag at the C- or N-terminus of the expressed mouse zcytor10 polypeptide, for example, a Glu-Glu epitope tag (Grussenmeyer, T. et al., Proc. Natl. Acad. Sci. 82:7952-4, 1985). Using a technique known in the art, a transfer vector containing mouse zcytor10 is transformed into E. Coli, and screened for bacmids which contain an interrupted lacZ gene indicative of recombinant baculovirus. The bacmid DNA containing the recombinant baculovirus genome is isolated, using common techniques, and used to transfect *Spodoptera frugiperda* cells, e.g. Sf9 cells. Recombinant virus that expresses mouse zcytor10 is subsequently produced. Recombinant viral stocks are made by methods commonly used in the art.

The recombinant virus is used to infect host cells, typically a cell line derived from the fall armyworm, *Spodoptera frugiperda*. See, in general, Glick and Pasternak, Molecular Biotechnology: Principles and Applications of Recombinant DNA, ASM Press, Washington, D.C., 1994. Another suitable cell line is the High FiveO™ cell line (Invitrogen) derived from *Trichoplusia ni* (U.S. Patent No. 5,300,435). Commercially available serum-free media are used to grow and maintain the cells. Suitable media are Sf900 II™ (Life Technologies) or ESF 921™ (Expression Systems) for the Sf9 cells; and Ex-cellO405™ (JRH Biosciences, Lenexa, KS) or Express FiveO™ (Life Technologies) for the *T. ni* cells. Procedures used are generally described in available laboratory manuals (King, L. A. and Possee, R.D., ibid.; O'Reilly, D.R. et al., ibid.; Richardson, C. D., ibid.). Subsequent purification of the mouse zcytor10 polypeptide from the supernatant can be achieved using methods described herein.

Fungal cells, including yeast cells, can also be used within the present invention. Yeast species of particular interest in this regard include *Saccharomyces cerevisiae*, *Pichia pastoris*, and *Pichia methanolica*. Methods for transforming *S. cerevisiae* cells with exogenous DNA and producing recombinant polypeptides therefrom are disclosed by, for example, Kawasaki, U.S. Patent No. 4,599,311; Kawasaki et al., U.S. Patent No. 4,931,373; Brake, U.S. Patent No. 4,870,008; Welch et al., U.S. Patent No. 5,037,743; and Murray et al., U.S. Patent No. 4,845,075. Transformed cells are selected by phenotype determined by the selectable marker, commonly drug resistance or the ability to grow in the absence of a particular nutrient (e.g., leucine). A preferred vector system for use in *Saccharomyces cerevisiae* is the *POT1* vector system disclosed by Kawasaki et al. (U.S. Patent No. 4,931,373), which allows transformed cells to be selected by growth in glucose-containing media. Suitable promoters and terminators for use in yeast include those from glycolytic enzyme genes (see, e.g., Kawasaki, U.S. Patent No. 4,599,311; Kingsman et al., U.S. Patent No. 4,615,974; and Bitter, U.S. Patent No. 4,977,092) and alcohol dehydrogenase genes. See also U.S. Patents Nos. 4,990,446; 5,063,154; 5,139,936 and 4,661,454. Transformation systems for other yeasts, including *Hansenula polymorpha, Schizosaccharomyces pombe, Kluyveromyces lactis, Kluyveromyces fragilis, Ustilago*

maydis, Pichia pastoris, Pichia methanolica, Pichia guillermondii and *Candida maltosa* are known in the art. See, for example, Gleeson et al., J. Gen. Microbiol. 132:3459-3465, 1986 and Cregg, U.S. Patent No. 4,882,279. *Aspergillus* cells may be utilized according to the methods of McKnight et al., U.S. Patent No. 4,935,349. Methods for transforming *Acremonium chrysogenum* are disclosed by Sumino et al., U.S. Patent No. 5,162,228. Methods for transforming *Neurospora* are disclosed by Lambowitz, U.S. Patent No. 4.486,533.

The use of *Pichia methanolica* as host for the production of recombinant proteins is disclosed in WIPO Publications WO 97/17450, WO 97/17451, WO 98/02536, and WO 98/02565. DNA molecules for use in transforming *P. methanolica* will commonly be prepared as double-stranded, circular plasmids, which are preferably linearized prior to transformation. For polypeptide production in *P. methanolica*, it is preferred that the promoter and terminator in the plasmid be that of a *P. methanolica* gene, such as a *P. methanolica* alcohol utilization gene (*AUG1* or *AUG2*). Other useful promoters include those of the dihydroxyacetone synthase (DHAS), formate dehydrogenase (FMD), and catalase (CAT) genes. To facilitate integration of the DNA into the host chromosome, it is preferred to have the entire expression segment of the plasmid flanked at both ends by host DNA sequences. A preferred selectable marker for use in *Pichia methanolica* is a *P. methanolica ADE2* gene, which encodes phosphoribosyl-5-aminoimidazole carboxylase (AIRC; EC 4.1.1.21), which allows *ade2* host cells to grow in the absence of adenine. For large-scale, industrial processes where it is desirable to minimize the use of methanol, it is preferred to use host cells in which both methanol utilization genes (*AUG1* and *AUG2*) are deleted. For production of secreted proteins, host cells deficient in vacuolar protease genes (*PEP4* and *PRB1*) are preferred. Electroporation is used to facilitate the introduction of a plasmid containing DNA encoding a polypeptide of interest into *P. methanolica* cells. It is preferred to transform *P. methanolica* cells by electroporation using an exponentially decaying, pulsed electric field having a field strength of from 2.5 to 4.5 kV/cm, preferably about 3.75 kV/cm, and a time constant (t) of from 1 to 40 milliseconds, most preferably about 20 milliseconds.

Prokaryotic host cells, including strains of the bacteria *Escherichia coli*, *Bacillus* and other genera are also useful host cells within the present invention. Techniques for transforming these hosts and expressing foreign DNA sequences cloned therein are well known in the art (see, e.g., Sambrook et al., ibid.). When expressing a mouse zcytor10 polypeptide in bacteria such as *E. coli*, the polypeptide may be retained in the cytoplasm, typically as insoluble granules, or may be directed to the periplasmic space by a bacterial secretion sequence. In the former case, the cells are lysed, and the granules are recovered and denatured using, for example, guanidine isothiocyanate or urea. The denatured polypeptide can then be refolded and dimerized by diluting the denaturant, such as by dialysis against a solution of urea and a combination of reduced and oxidized glutathione, followed by dialysis against a buffered saline solution. In the latter case, the polypeptide can be recovered from the periplasmic space in a soluble and functional form by disrupting the cells (by, for example, sonication or osmotic shock) to release the contents of the periplasmic space and recovering the protein, thereby obviating the need for denaturation and refolding.

Transformed or transfected host cells are cultured according to conventional procedures in a culture medium containing nutrients and other components required for the growth of the chosen host cells. A variety of suitable media, including defined media and complex media, are known in the art and generally include a carbon source, a nitrogen source, essential amino acids, vitamins and minerals. Media may also contain such components as growth factors or serum, as required. The growth medium will generally select for cells containing the exogenously added DNA by, for example, drug selection or deficiency in an essential nutrient which is complemented by the selectable marker carried on the expression vector or co-transfected into the host cell. *P. methanolica* cells are cultured in a medium comprising adequate sources of carbon, nitrogen and trace nutrients at a temperature of about 25°C to 35°C. Liquid cultures are provided with sufficient aeration by conventional means, such as shaking of small flasks or sparging of fermentors. A preferred culture medium for *P. methanolica* is YEPD (2% D-glucose, 2% Bacto™ Peptone (Difco Laboratories, Detroit, MI), 1% Bacto™ yeast extract (Difco Laboratories), 0.004% adenine and 0.006% L-leucine).

Within one aspect of the present invention, a mouse zcytor10 cytokine receptor (including transmembrane and intracellular domains) is produced by a cultured cell, and the cell is used to screen for ligands for the receptor, including the natural ligand, as well as agonists and antagonists of the natural ligand. To summarize this approach, a cDNA or gene encoding the receptor is combined with other genetic elements required for its expression (e.g., a transcription promoter), and the resulting expression vector is inserted into a host cell. Cells that express the DNA and produce functional receptor are selected and used within a variety of screening systems.

Mammalian cells suitable for use in expressing the novel receptors of the present invention and transducing a receptor-mediated signal include cells that express a β-subunit, such as gp130, and cells that co-express gp130 and LIF receptor (Gearing et al., EMBO J. 10:2839-2848, 1991; Gearing et al., U.S. Patent No. 5,284,755). In this regard it is generally preferred to employ a cell that is responsive to other cytokines that bind to receptors in the same subfamily, such as IL-6 or LIF, because such cells will contain the requisite signal transduction pathway(s). Preferred cells of this type include the human TF-1 cell line (ATCC number CRL-2003) and the DA-1 cell line (Branch et al., Blood 69:1782, 1987; Broudy et al., Blood 75:1622-1626, 1990). In the alternative, suitable host cells can be engineered to produce a β-subunit or other cellular component needed for the desired cellular response. For example, the murine cell line BaF3 (Palacios and Steinmetz, Cell 41:727-734, 1985; Mathey-Prevot et al., Mol. Cell. Biol. 6: 4133-4135, 1986), a baby hamster kidney (BHK) cell line, or the CTLL-2 cell line (ATCC TIB-214) can be transfected to express the mouse gp130 subunit, or mouse gp130 and LIF receptor, in addition to mouse zcytor10. It is generally preferred to use a host cell and receptor(s) from the same species, however this approach allows cell lines to be engineered to express multiple receptor subunits from any species, thereby overcoming potential limitations arising from species specificity. In the alternative, species homologs of the mouse receptor cDNA can be cloned and used within cell lines from the same species, such as a mouse cDNA in the BaF3 cell line. Cell lines that are dependent upon one hematopoietic growth factor, such as IL-3, can thus be engineered to become dependent upon a mouse zcytor10 ligand.

Cells expressing functional mouse zcytor10 are used within screening assays. A variety of suitable routine assays are high throughput and well known in the art. These assays are based on the detection of a biological response in the target cell. One such assay is a cell proliferation assay. Cells are cultured in the presence or absence of a test compound, and cell proliferation is detected by, for example, measuring incorporation of tritiated thymidine or by colorimetric assay based on the metabolic breakdown of Alymar Blue™ (AccuMed, Chicago, IL) or 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) (Mosman, J. Immunol. Meth. 65: 55-63, 1983). An alternative assay format uses cells that are further engineered to express a reporter gene. The reporter gene is linked to a promoter element that is responsive to the receptor-linked pathway, and the assay detects activation of transcription of the reporter gene. A preferred promoter element in this regard is a serum response element, or SRE (see, for example, Shaw et al., Cell 56:563-572, 1989). A preferred such reporter gene is a luciferase gene (de Wet et al., Mol. Cell. Biol. 7:725, 1987). Expression of the luciferase gene is detected by luminescence using methods known in the art (e.g., Baumgartner et al., J. Biol. Chem. 269:19094-29101, 1994; Schenborn and Goiffin, Promega Notes 41:11, 1993). Luciferase assay kits are commercially available from, for example, Promega Corp., Madison, WI. Target cell lines of this type can be used to screen libraries of chemicals, cell-conditioned culture media, fungal broths, soil samples, water samples, and the like. For example, a bank of cell- or tissue-conditioned media samples can be assayed on a target cell to identify cells that produce ligand. Positive cells are then used to produce a cDNA library in a mammalian cell expression vector, which is divided into pools, transfected into host cells, and expressed. Media samples from the transfected cells are then assayed, with subsequent division of pools, retransfection, subculturing, and re-assay of positive cells to isolate a clonal cell line expressing the ligand. Media samples conditioned by kidney, liver, spleen, thymus, other lymphoid tissues, or T-cells are preferred sources of ligand for use in screening procedures.

A natural ligand for mouse zcytor10 can also be identified by mutagenizing a cytokine-dependent cell line expressing mouse zcytor10 and culturing it under conditions that select for autocrine growth. See WIPO publication WO

95/21930. Within a typical procedure, cells expressing mouse zcytor10 are mutagenized, such as with EMS. The cells are then allowed to recover in the presence of the required cytokine, then transferred to a culture medium lacking the cytokine. Surviving cells are screened for the production of a ligand for mouse zcytor10, such as by adding soluble (ligand-binding) receptor polypeptide to the culture medium or by assaying conditioned media on wild-type cells and transfected cells expressing the mouse zcytor10. Preferred cell lines for use within this method include cells that are transfected to express gp130 or gp130 in combination with LIF receptor. Preferred such host cell lines include transfected CTLL-2 cells (Gillis and Smith, Nature 268:154-156, 1977) and transfected BaF3 cells.

Moreover, a secretion trap method employing mouse zcytor10 soluble receptor polypeptide can be used to isolate a mouse zcytor10 ligand (Aldrich, et al, Cell 87: 1161-1169, 1996). A cDNA expression library prepared from a known or suspected ligand source is transfected into COS-7 cells. The cDNA library vector generally has an SV40 origin for amplification in COS-7 cells, and a CMV promoter for high expression. The transfected COS-7 cells are grown in a monolayer and then fixed and permeabilized. Tagged or biotin-labeled mouse zcytor10 soluble receptor, described herein, is then placed in contact with the cell layer and allowed to bind cells in the monolayer that express an anti-complementary molecule, i.e., a mouse zcytor10 ligand. A cell expressing a ligand will thus be bound with receptor molecules. An anti-tag antibody (anti-Ig for Ig fusions, M2 or anti-FLAG for FLAG-tagged fusions, streptavidin, and the like) which is conjugated with horseradish peroxidase (HRP) is used to visualize these cells to which the tagged or biotin-labeled mouse zcytor10 soluble receptor has bound. The HRP catalyzes deposition of a tyramide reagent, for example, tyramide-FITC. A commercially-available kit can be used for this detection (for example, Renaissance TSA-Direct™ Kit; NEN Life Science Products, Boston, MA). Cells which express mouse zcytor10 receptor ligand will be identified under fluorescence microscopy as green cells and picked for subsequent cloning of the ligand using procedures for plasmid rescue as outlined in Aldrich, et al, supra., followed by subsequent rounds of secretion trap assay until single clones are identified.

As a receptor, the activity of mouse zcytor10 polypeptide can be measured by a silicon-based biosensor microphysiometer which measures the extracellular acidification rate or proton excretion associated with receptor binding and subsequent physiologic cellular responses. An exemplary device is the Cytosensor™ Microphysiometer manufactured by Molecular Devices, Sunnyvale, CA. A variety of cellular responses, such as cell proliferation, ion transport, energy production, inflammatory response, regulatory and receptor activation, and the like, can be measured by this method. See, for example, McConnell, H.M. et al., Science 257:1906-1912, 1992; Pitchford, S. et al., Meth. Enzymol. 228:84-108, 1997; Arimilli, S. et al., J. Immunol. Meth. 212:49-59, 1998; Van Liefde, I. Et al., Eur. J. Pharmacol. 346:87-95, 1998. The microphysiometer can be used for assaying eukaryotic, prokaryotic, adherent or non-adherent cells. By measuring extracellular acidification changes in cell media over time, the microphysiometer directly measures cellular responses to various stimuli, including agonists, ligands, or antagonists of the mouse zcytor10 polypeptide. Preferably, the microphysiometer is used to measure responses of a mouse zcytor10-expressing eukaryotic cell, compared to a control eukaryotic cell that does not express mouse zcytor10 polypeptide. Mouse zcytor10-expressing eukaryotic cells comprise cells into which mouse zcytor10 has been transfected, as described herein, creating a cell that is responsive to mouse zcytor10-modulating stimuli, or are cells naturally expressing mouse zcytor10, such as mouse zcytor10-expressing cells derived from lymphoid, spleen, thymus tissue, PBLs, lung, liver, heart or testis. Differences, measured by an increase or decrease in extracellular acidification, in the response of cells expressing mouse zcytor10, relative to a control, are a direct measurement of mouse zcytor10-modulated cellular responses. Moreover, such mouse zcytor10-modulated responses can be assayed under a variety of stimuli. Also, using the microphysiometer, there is provided a method of identifying agonists and antagonists of mouse zcytor10 polypeptide, comprising providing cells expressing a mouse zcytor10 polypeptide, culturing a first portion of the cells in the absence of a test compound, culturing a second portion of the cells in the presence of a test compound, and detecting an increase or a decrease in a cellular response of the second portion of the cells as compared to the first portion of the cells. Antagonists and agonists,

including the natural ligand for zcytor10 polypeptide, can be rapidly identified using this method.

Additional assays provided by the present invention include the use of hybrid receptor polypeptides. These hybrid polypeptides fall into two general classes. Within the first class, the intracellular domain of mouse zcytor10, comprising approximately residues 252 (Arg) to 357 (Leu) of SEQ ID NO:2, or residues 254 (Arg) to 359 (Leu) of SEQ ID NO:35, is joined to the ligand-binding domain of a second receptor. It is preferred that the second receptor be a hematopoietic cytokine receptor, such as mpl receptor (Souyri et al., Cell 63:1137-1147, 1990). The hybrid receptor will further comprise a transmembrane domain, which may be derived from either receptor. A DNA construct encoding the hybrid receptor is then inserted into a host cell. Cells expressing the hybrid receptor are cultured in the presence of a ligand for the binding domain and assayed for a response. This system provides a means for analyzing signal transduction mediated by mouse zcytor10 while using readily available ligands. This system can also be used to determine if particular cell lines are capable of responding to signals transduced by mouse zcytor10. A second class of hybrid receptor polypeptides comprise the extracellular (ligand-binding) domain of mouse zcytor10 (approximately residues 15 (Cys) to 230 (Pro) of SEQ ID NO:2, or 17 (Ala) to 232 (Pro) of SEQ ID NO:35) with a cytoplasmic domain of a second receptor, preferably a cytokine receptor, and a transmembrane domain. The transmembrane domain may be derived from either the mouse zcytor10 receptor or second receptor. Hybrid receptors of this second class are expressed in cells known to be capable of responding to signals transduced by the second receptor. Together, these two classes of hybrid receptors enable the use of a broad spectrum of cell types within receptor-based assay systems.

Cells found to express a ligand for mouse zcytor10 are then used to prepare a cDNA library from which the ligand-encoding cDNA may be isolated as disclosed above. The present invention thus provides, in addition to novel receptor polypeptides, methods for cloning polypeptide ligands for the receptors.

The mouse zcytor10 may play a role in early thymocyte development and/or immune response regulation. These processes involve stimulation of cell proliferation and differentiation in response to the binding of one or more cytokines to

their cognate receptors. In view of the tissue distribution observed for this receptor, agonists (including the natural ligand) and antagonists have enormous potential in both *in vitro* and *in vivo* applications. Compounds identified as receptor agonists are useful for stimulating proliferation and development of target cells *in vitro* and *in vivo*. For example, agonist compounds are useful as components of defined cell culture media, and may be used alone or in combination with other cytokines and hormones to replace serum that is commonly used in cell culture. Agonists are thus useful in specifically promoting the growth and/or development of T-cells, B-cells, and other cells of the lymphoid and myeloid lineages, and hematopoietic cells in culture. Assays for determining growth and development of these cell lineages are well known in the art.

Agonist ligands for mouse zcytor10 may be useful in stimulating cell-mediated immunity and for stimulating lymphocyte proliferation, such as in a mouse model for use in studying the treatment of infections involving immunosuppression, including certain viral infections. Additional uses include use in a mouse model for studying tumor suppression, where malignant transformation results in tumor cells that are antigenic. Agonist ligands could be used to induce cytotoxicity, which may be mediated through activation of effector cells such as T-cells, NK (natural killer) cells, or LAK (lymphoid activated killer) cells, or induced directly through apoptotic pathways, ans as such applied in mouse models for human disease. Agonist ligands may also be useful in a mouse model for studying potential treatments for leukopenias by increasing the levels of the affected cell type, and for studies involving enhancing the regeneration of the T-cell repertoire after bone marrow transplantation.

Antagonist or agonist ligands or compounds may find utility in the suppression of the immune system, and provide a useful mouse model for studying the treatment of autoimmune diseases, including rheumatoid arthritis, multiple sclerosis, diabetes mellitis, inflammatory bowel disease, Crohn's disease, etc. Immune suppression can also be used to reduce rejection of tissue or organ transplants and grafts and to treat T-cell specific leukemias or lymphomas by inhibiting proliferation of the affected cell type.

Mouse zcytor10 may also be used within diagnostic systems for the detection of circulating levels of both human and mouse ligand. Within a related

embodiment, antibodies or other agents that bind to mouse zcytor10 can be used to detect circulating receptor polypeptides. Elevated or depressed levels of ligand or receptor polypeptides may be indicative of pathological conditions, including cancer. Soluble receptor polypeptides may contribute to pathologic processes and can be an indirect marker of an underlying disease; as such, a mouse model expressing mouse zcytor10 soluble receptors can be utilized as a model to study a human pathologic process. For example, elevated levels of soluble IL-2 receptor in human serum have been associated with a wide variety of inflammatory and neoplastic conditions, such as myocardial infarction, asthma, myasthenia gravis, rheumatoid arthritis, acute T-cell leukemia, B-cell lymphomas, chronic lymphocytic leukemia, colon cancer, breast cancer, and ovarian cancer (Heaney et al., Blood 87:847-857, 1996).

A ligand-binding polypeptide of a mouse zcytor10 receptor, or "soluble receptor," can be prepared by expressing a truncated DNA encoding the mouse zcytor10 cytokine binding domain (approximately residue 15 (Cys) to 230 (Pro) of the murine receptor (SEQ ID NO:2); or approximately 17 (Ala) to 232 (Pro) of SEQ ID NO:35) or the corresponding region of a mouse paralog or non-mouse receptor. It is preferred that the extracellular domain be prepared in a form substantially free of transmembrane and intracellular polypeptide segments. Moreover, ligand-binding polypeptide fragments within the mouse zcytor10 cytokine binding domain, described above, can also serve as mouse zcytor10 soluble receptors for uses described herein. To direct the export of a receptor polypeptide from the host cell, the receptor DNA is linked to a second DNA segment encoding a secretory peptide, such as a t-PA secretory peptide or a mouse zcytor10 secretory peptide. To facilitate purification of the secreted receptor polypeptide, a C-terminal extension, such as a poly-histidine tag, substance P, Flag™ peptide (Hopp et al., Bio/Technology 6:1204-1210, 1988; available from Eastman Kodak Co., New Haven, CT) or another polypeptide or protein for which an antibody or other specific binding agent is available, can be fused to the receptor polypeptide.

In an alternative approach, a receptor extracellular domain can be expressed as a fusion with immunoglobulin heavy chain constant regions, typically an F_c fragment, which contains two constant region domains and lacks the variable region.

Such fusions are typically secreted as multimeric molecules wherein the Fc portions are disulfide bonded to each other and two receptor polypeptides are arrayed in close proximity to each other. Fusions of this type can be used to affinity purify the cognate ligand from solution, as an *in vitro* assay tool, to block signals *in vitro* by specifically titrating out ligand, and as antagonists *in vivo* by administering them parenterally to bind circulating ligand and clear it from the circulation. To purify ligand, a mouse zcytor10-Ig chimera is added to a sample containing the ligand (e.g., cell-conditioned culture media or tissue extracts) under conditions that facilitate receptor-ligand binding (typically near-physiological temperature, pH, and ionic strength). The chimera-ligand complex is then separated by the mixture using protein A, which is immobilized on a solid support (e.g., insoluble resin beads). The ligand is then eluted using conventional chemical techniques, such as with a salt or pH gradient. In the alternative, the chimera itself can be bound to a solid support, with binding and elution carried out as above. Collected fractions can be re-fractionated until the desired level of purity is reached.

Moreover, mouse zcytor10 soluble receptors can be used as a "ligand sink," i.e., antagonist, to bind ligand *in vivo* or *in vitro* in a murine model for therapeutic or other applications where the presence of the ligand is not desired. Similarly, the mouse zcytor10 soluble receptors can be used as an antagonist to bind human ligand *in vitro* or *in vivo* for therapeutic or other applications. For example, in cancers that are expressing large amount of bioactive zcytor10 ligand, mouse zcytor10 soluble receptors can be used as a direct antagonist of the ligand *in vivo*, and may aid in reducing progression and symptoms associated with the disease. Moreover, mouse zcytor10 soluble receptor can be used to slow the progression of cancers that over-express zcytor10 receptors, by binding ligand *in vivo* that would otherwise enhance proliferation of those cancers. Similar *in vitro* applications for a mouse zcytor10 soluble receptor can be used, for instance, as a negative selection to select cell lines that grow in the absence of mouse zcytor10 ligand.

Moreover, mouse zcytor10 soluble receptor can be used *in vivo* or in diagnostic applications to detect zcytor10 ligand-expressing cancers *in vivo* or in tissue samples, including human cancers and tissues that express a human orthologous ligand. For example, the mouse zcytor10 soluble receptor can be conjugated to a radio-label or

fluorescent label as described herein, and used to detect the presence of the human or mouse ligand in a tissue sample using an *in vitro* ligand-receptor type binding assay, or fluorescent imaging assay. Moreover, a radio-labeled mouse zcytor10 soluble receptor could be administered *in vivo* to detect ligand-expressing solid tumors through a radio-imaging method known in the art.

As a cytokine receptor, a role for the mouse zcytor10 receptor in proliferation, differentiation, and/or activation of immune cells, and in development and regulation of immune responses is suggested. The interaction of mouse zcytor10 with its ligand may stimulate proliferation and development of myeloid cells and may, like IL-2, IL-6, LIF, IL-11 and OSM (Baumann et al., J. Biol. Chem. 268:8414-8417, 1993), induce acute-phase protein synthesis in hepatocytes.

It is preferred to purify the polypeptides of the present invention to ≥80% purity, more preferably to ≥90% purity, even more preferably ≥95% purity, and particularly preferred is a pharmaceutically pure state, that is greater than 99.9% pure with respect to contaminating macromolecules, particularly other proteins and nucleic acids, and free of infectious and pyrogenic agents. Preferably, a purified polypeptide is substantially free of other polypeptides, particularly other polypeptides of animal origin.

Expressed recombinant mouse zcytor10 polypeptides (or mouse zcytor10 chimeric or fusion polypeptides) can be purified using fractionation and/or conventional purification methods and media. Ammonium sulfate precipitation and acid or chaotrope extraction may be used for fractionation of samples. Exemplary purification steps may include hydroxyapatite, size exclusion, FPLC and reverse-phase high performance liquid chromatography. Suitable chromatographic media include derivatized dextrans, agarose, cellulose, polyacrylamide, specialty silicas, and the like. PEI, DEAE, QAE and Q derivatives are preferred. Exemplary chromatographic media include those media derivatized with phenyl, butyl, or octyl groups, such as Phenyl-Sepharose FF (Pharmacia), Toyopearl butyl 650 (Toso Haas, Montgomeryville, PA), Octyl-Sepharose (Pharmacia) and the like; or polyacrylic resins, such as Amberchrom CG 71 (Toso Haas) and the like. Suitable solid supports include glass beads, silica-based resins, cellulosic resins, agarose beads, cross-linked agarose beads, polystyrene beads, cross-linked polyacrylamide resins and the like that are insoluble under the

conditions in which they are to be used. These supports may be modified with reactive groups that allow attachment of proteins by amino groups, carboxyl groups, sulfhydryl groups, hydroxyl groups and/or carbohydrate moieties. Examples of coupling chemistries include cyanogen bromide activation, N-hydroxysuccinimide activation, epoxide activation, sulfhydryl activation, hydrazide activation, and carboxyl and amino derivatives for carbodiimide coupling chemistries. These and other solid media are well known and widely used in the art, and are available from commercial suppliers. Methods for binding receptor polypeptides to support media are well known in the art. Selection of a particular method is a matter of routine design and is determined in part by the properties of the chosen support. See, for example, Affinity Chromatography: Principles & Methods, Pharmacia LKB Biotechnology, Uppsala, Sweden, 1988.

The polypeptides of the present invention can be isolated by exploitation of their biochemical, structural, and biological properties. For example, immobilized metal ion adsorption (IMAC) chromatography can be used to purify histidine-rich proteins, including those comprising polyhistidine tags. Briefly, a gel is first charged with divalent metal ions to form a chelate (Sulkowski, Trends in Biochem. 3:1-7, 1985). Histidine-rich proteins will be adsorbed to this matrix with differing affinities, depending upon the metal ion used, and will be eluted by competitive elution, lowering the pH, or use of strong chelating agents. Other methods of purification include purification of glycosylated proteins by lectin affinity chromatography and ion exchange chromatography (Methods in Enzymol., Vol. 182, "Guide to Protein Purification", M. Deutscher, (ed.), Acad. Press, San Diego, 1990, pp.529-39). Within additional embodiments of the invention, a fusion of the polypeptide of interest and an affinity tag (e.g., maltose-binding protein, an immunoglobulin domain) may be constructed to facilitate purification.

Moreover, using methods described in the art, polypeptide fusions, or hybrid mouse zcytor10 proteins, are constructed using regions or domains of the inventive mouse zcytor10 in combination with those of other mouse or human cytokine receptor family proteins, or heterologous proteins (Sambrook et al., ibid., Altschul et al., ibid., Picard, Cur. Opin. Biology, 5:511-5, 1994, and references therein). These methods allow the determination of the biological importance of larger domains or

regions in a polypeptide of interest. Such hybrids may alter reaction kinetics, binding, constrict or expand the substrate specificity, or alter tissue and cellular localization of a polypeptide, and can be applied to polypeptides of unknown structure.

Fusion polypeptides or proteins can be prepared by methods known to those skilled in the art by preparing each component of the fusion protein and chemically conjugating them. Alternatively, a polynucleotide encoding one or more components of the fusion protein in the proper reading frame can be generated using known techniques and expressed by the methods described herein. For example, part or all of a domain(s) conferring a biological function may be swapped between mouse zcytor10 of the present invention with the functionally equivalent domain(s) from another cytokine family member. Such domains include, but are not limited to, the secretory signal sequence, extracellular cytokine binding domain, transmembrane domain, and intracellular signaling domain, Box I and Box II sites, block 1, mammalian signaling motif, and motifs 1-6, as disclosed herein. Such fusion proteins would be expected to have a biological functional profile that is the same or similar to polypeptides of the present invention or other known family proteins, depending on the fusion constructed. Moreover, such fusion proteins may exhibit other properties as disclosed herein.

Standard molecular biological and cloning techniques can be used to swap the equivalent domains between the mouse zcytor10 polypeptide and those polypeptides to which they are fused (e.g., human zcytor10 or other cytokine receptors). Generally, a DNA segment that encodes a domain of interest, e.g., a mouse zcytor10 domain described herein, is operably linked in frame to at least one other DNA segment encoding an additional polypeptide (for instance a domain or region from another cytokine receptor, such as IL-7R, IL-3R, IL2R, EPO receptor, or the like), and inserted into an appropriate expression vector, as described herein. Generally DNA constructs are made such that the several DNA segments that encode the corresponding regions of a polypeptide are operably linked in frame to make a single construct that encodes the entire fusion protein, or a functional portion thereof. For example, a DNA construct would encode from N-terminus to C-terminus a fusion protein comprising a signal polypeptide followed by a cytokine binding domain, followed by a transmembrane

domain, followed by an intracellular signaling domain. Such fusion proteins can be expressed, isolated, and assayed for activity as described herein.

Mouse zcytor10 polypeptides or fragments thereof may also be prepared through chemical synthesis. mouse zcytor10 polypeptides may be monomers or multimers; glycosylated or non-glycosylated; pegylated or non-pegylated; and may or may not include an initial methionine amino acid residue.

Polypeptides of the present invention can also be synthesized by exclusive solid phase synthesis, partial solid phase methods, fragment condensation or classical solution synthesis. Methods for synthesizing polypeptides are well known in the art. See, for example, Merrifield, J. Am. Chem. Soc. 85:2149, 1963; Kaiser et al., Anal. Biochem. 34:595, 1970. After the entire synthesis of the desired peptide on a solid support, the peptide-resin is with a reagent which cleaves the polypeptide from the resin and removes most of the side-chain protecting groups. Such methods are well established in the art.

The activity of molecules of the present invention can be measured using a variety of assays that measure cell differentiation and proliferation. Such assays are well known in the art.

Proteins of the present invention are useful for example, in treating lymphoid, immune, inflammatory, spleenic, blood or bone disorders, and can be measured *in vitro* using cultured cells or *in vivo* by administering molecules of the claimed invention to the appropriate animal model. For instance, host cells expressing a zcytor10 soluble receptor polypeptide can be embedded in an alginate environment and injected (implanted) into recipient animals. Alginate-poly-L-lysine microencapsulation, permselective membrane encapsulation and diffusion chambers are a means to entrap transfected mammalian cells or primary mammalian cells. These types of non-immunogenic "encapsulations" permit the diffusion of proteins and other macromolecules secreted or released by the captured cells to the recipient animal. Most importantly, the capsules mask and shield the foreign, embedded cells from the recipient animal's immune response. Such encapsulations can extend the life of the injected cells from a few hours or days (naked cells) to several weeks (embedded cells). Alginate threads provide a simple and quick means for generating embedded cells.

The materials needed to generate the alginate threads are known in the art. In an exemplary procedure, 3% alginate is prepared in sterile H_2O, and sterile filtered. Just prior to preparation of alginate threads, the alginate solution is again filtered. An approximately 50% cell suspension (containing about 5 x 10^5 to about 5 x 10^7 cells/ml) is mixed with the 3% alginate solution. One ml of the alginate/cell suspension is extruded into a 100 mM sterile filtered $CaCl_2$ solution over a time period of ~15 min, forming a "thread". The extruded thread is then transferred into a solution of 50 mM $CaCl_2$, and then into a solution of 25 mM $CaCl_2$. The thread is then rinsed with deionized water before coating the thread by incubating in a 0.01% solution of poly-L-lysine. Finally, the thread is rinsed with Lactated Ringer's Solution and drawn from solution into a syringe barrel (without needle). A large bore needle is then attached to the syringe, and the thread is intraperitoneally injected into a recipient in a minimal volume of the Lactated Ringer's Solution.

An *in vivo* approach for assaying proteins of the present invention involves viral delivery systems. Exemplary viruses for this purpose include adenovirus, herpesvirus, retroviruses, vaccinia virus, and adeno-associated virus (AAV). Adenovirus, a double-stranded DNA virus, is currently the best studied gene transfer vector for delivery of heterologous nucleic acid (for review, see T.C. Becker et al., Meth. Cell Biol. 43:161-89, 1994; and J.T. Douglas and D.T. Curiel, Science & Medicine 4:44-53, 1997). The adenovirus system offers several advantages: (i) adenovirus can accommodate relatively large DNA inserts; (ii) can be grown to high-titer; (iii) infect a broad range of mammalian cell types; and (iv) can be used with a large number of different promoters including ubiquitous, tissue specific, and regulatable promoters. Also, because adenoviruses are stable in the bloodstream, they can be administered by intravenous injection.

Using adenovirus vectors where portions of the adenovirus genome are deleted, inserts are incorporated into the viral DNA by direct ligation or by homologous recombination with a co-transfected plasmid. In an exemplary system, the essential E1 gene has been deleted from the viral vector, and the virus will not replicate unless the E1 gene is provided by the host cell (the human 293 cell line is exemplary). When intravenously administered to intact animals, adenovirus primarily targets the liver. If

the adenoviral delivery system has an E1 gene deletion, the virus cannot replicate in the host cells. However, the host's tissue (e.g., liver) will express and process (and, if a secretory signal sequence is present, secrete) the heterologous protein. Secreted proteins will enter the circulation in the highly vascularized liver, and effects on the infected animal can be determined.

Moreover, adenoviral vectors containing various deletions of viral genes can be used in an attempt to reduce or eliminate immune responses to the vector. Such adenoviruses are E1 deleted, and in addition contain deletions of E2A or E4 (Lusky, M. et al., J. Virol. 72:2022-2032, 1998; Raper, S.E. et al., Human Gene Therapy 9:671-679, 1998). In addition, deletion of E2b is reported to reduce immune responses (Amalfitano, A. et al., J. Virol. 72:926-933, 1998). Moreover, by deleting the entire adenovirus genome, very large inserts of heterologous DNA can be accommodated. Generation of so called "gutless" adenoviruses where all viral genes are deleted are particularly advantageous for insertion of large inserts of heterologous DNA. For review, see Yeh, P. and Perricaudet, M., FASEB J. 11:615-623, 1997.

The adenovirus system can also be used for protein production *in vitro*. By culturing adenovirus-infected non-293 cells under conditions where the cells are not rapidly dividing, the cells can produce proteins for extended periods of time. For instance, BHK cells are grown to confluence in cell factories, then exposed to the adenoviral vector encoding the secreted protein of interest. The cells are then grown under serum-free conditions, which allows infected cells to survive for several weeks without significant cell division. Alternatively, adenovirus vector infected 293 cells can be grown as adherent cells or in suspension culture at relatively high cell density to produce significant amounts of protein (See Garnier et al., Cytotechnol. 15:145-55, 1994). With either protocol, an expressed, secreted heterologous protein can be repeatedly isolated from the cell culture supernatant, lysate, or membrane fractions depending on the disposition of the expressed protein in the cell. Within the infected 293 cell production protocol, non-secreted proteins may also be effectively obtained.

In view of the class I cytokine structure observed for mouse zcytor10, agonists (including the natural ligand/ substrate/ cofactor/ etc.) and antagonists have enormous potential in both *in vitro* and *in vivo* applications. Compounds identified as

mouse zcytor10 agonists are useful for stimulating growth of immune and hematopoietic cells *in vitro* and *in vivo*. For example, mouse zcytor10 and agonist compounds are useful as components of defined cell culture media, and may be used alone or in combination with other cytokines and hormones to replace serum that is commonly used in cell culture. Agonists are thus useful in specifically promoting the growth and/or development of T-cells, B-cells, and other cells of the lymphoid and myeloid lineages in culture. Moreover, mouse zcytor10 soluble receptor, agonist, or antagonist may be used in vitro in an assay to measure stimulation of colony formation from isolated primary bone marrow cultures. Such assays are well known in the art.

Antagonists are also useful as research reagents for characterizing sites of ligand-receptor interaction. Inhibitors of mouse zcytor10 activity (mouse zcytor10 antagonists) include anti-mouse zcytor10 antibodies and soluble mouse zcytor10 receptors, as well as other peptidic and non-peptidic agents (including ribozymes).

Mouse zcytor10 can also be used to identify modulators (e.g, antagonists) of its activity. Test compounds are added to the assays disclosed herein to identify compounds that inhibit the activity of mouse zcytor10. In addition to those assays disclosed herein, samples can be tested for inhibition of mouse zcytor10 activity within a variety of assays designed to measure mouse zcytor10 binding, oligomerization, or the stimulation/inhibition of mouse zcytor10-dependent cellular responses. For example, mouse zcytor10-expressing cell lines can be transfected with a reporter gene construct that is responsive to a mouse zcytor10-stimulated cellular pathway. Reporter gene constructs of this type are known in the art, and will generally comprise a mouse zcytor10-DNA response element operably linked to a gene encoding an assay detectable protein, such as luciferase. DNA response elements can include, but are not limited to, cyclic AMP response elements (CRE), hormone response elements (HRE) insulin response element (IRE) (Nasrin et al., Proc. Natl. Acad. Sci. USA 87:5273-7, 1990) and serum response elements (SRE) (Shaw et al. Cell 56: 563-72, 1989). Cyclic AMP response elements are reviewed in Roestler et al., J. Biol. Chem. 263 (19):9063-6; 1988 and Habener, Molec. Endocrinol. 4 (8):1087-94; 1990. Hormone response elements are reviewed in Beato, Cell 56:335-44; 1989. Candidate compounds, solutions, mixtures or extracts or conditioned media from various cell

types are tested for the ability to enhance the activity of mouse zcytor10 receptor as evidenced by a increase in mouse zcytor10 stimulation of reporter gene expression. Assays of this type will detect compounds that directly stimulate mouse zcytor10 signal transduction activity through binding the receptor or by otherwise stimulating part of the signal cascade. As such, there is provided a method of identifying agonists of mouse zcytor10 polypeptide, comprising providing cells responsive to a mouse zcytor10 polypeptide, culturing a first portion of the cells in the absence of a test compound, culturing a second portion of the cells in the presence of a test compound, and detecting a increase in a cellular response of the second portion of the cells as compared to the first portion of the cells. Moreover third cell, containing the reporter gene construct described above, but not expressing zaplpha11 receptor, can be used as a control cell to assess non-specific, or non-mouse zcytor10-mediated, stimulation of the reporter. Agonists, including the natural ligand, are therefore useful to stimulate or increase mouse zcytor10 polypeptide function.

A mouse zcytor10 ligand-binding polypeptide, such as the cytokine binding domain disclosed herein, can also be used for purification of ligand. The polypeptide is immobilized on a solid support, such as beads of agarose, cross-linked agarose, glass, cellulosic resins, silica-based resins, polystyrene, cross-linked polyacrylamide, or like materials that are stable under the conditions of use. Methods for linking polypeptides to solid supports are known in the art, and include amine chemistry, cyanogen bromide activation, N-hydroxysuccinimide activation, epoxide activation, sulfhydryl activation, and hydrazide activation. The resulting medium will generally be configured in the form of a column, and fluids containing ligand are passed through the column one or more times to allow ligand to bind to the receptor polypeptide. The ligand is then eluted using changes in salt concentration, chaotropic agents (guanidine HCl), or pH to disrupt ligand-receptor binding.

An assay system that uses a ligand-binding receptor (or an antibody, one member of a complement/ anti-complement pair) or a binding fragment thereof, and a commercially available biosensor instrument may be advantageously employed (e.g., BIAcore™, Pharmacia Biosensor, Piscataway, NJ; or SELDI™ technology, Ciphergen, Inc., Palo Alto, CA). Such receptor, antibody, member of a complement/anti-

complement pair or fragment is immobilized onto the surface of a receptor chip. Use of this instrument is disclosed by Karlsson, J. Immunol. Methods 145:229-240, 1991 and Cunningham and Wells, J. Mol. Biol. 234:554-63, 1993. A receptor, antibody, member or fragment is covalently attached, using amine or sulfhydryl chemistry, to dextran fibers that are attached to gold film within the flow cell. A test sample is passed through the cell. If a ligand, epitope, or opposite member of the complement/anti-complement pair is present in the sample, it will bind to the immobilized receptor, antibody or member, respectively, causing a change in the refractive index of the medium, which is detected as a change in surface plasmon resonance of the gold film. This system allows the determination of on- and off-rates, from which binding affinity can be calculated, and assessment of stoichiometry of binding.

Ligand-binding receptor polypeptides can also be used within other assay systems known in the art. Such systems include Scatchard analysis for determination of binding affinity (see Scatchard, Ann. NY Acad. Sci. 51: 660-672, 1949) and calorimetric assays (Cunningham et al., Science 253:545-48, 1991; Cunningham et al., Science 245:821-25, 1991).

Mouse zcytor10 polypeptides can also be used to prepare antibodies that bind to mouse zcytor10 epitopes, peptides or polypeptides. The mouse zcytor10 polypeptide or a fragment thereof serves as an antigen (immunogen) to inoculate an animal and elicit an immune response. One of skill in the art would recognize that antigenic, epitope-bearing polypeptides contain a sequence of at least 6, preferably at least 9, and more preferably at least 15 to about 30 contiguous amino acid residues of a zcytor10 polypeptide (e.g., SEQ ID NO:2). Polypeptides comprising a larger portion of a zcytor10 polypeptide, i.e., from 10 to 30 residues up to the entire length of the amino acid sequence are included. Antigens or immunogenic epitopes can also include attached tags, adjuvants and carriers, as described herein. Suitable antigens include the mouse zcytor10 polypeptide encoded by SEQ ID NO:2 from amino acid number 15 (Cys) to amino acid number 357 (Leu), or a contiguous 9 to 343 amino acid fragment thereof. Similarly, suitable antigens include the mouse zcytor10 polypeptide encoded by SEQ ID NO:2 from amino acid number 17 (Ala) to amino acid number 359 (Leu), or

a contiguous 9 to 343 amino acid fragment thereof. Preferred peptides to use as antigens are the cytokine binding domain, intracellular signaling domain, Box I and Box II sites, block 1, mammalian signaling motif, and motifs 1-6, disclosed herein, and mouse zcytor10 hydrophilic peptides such as those predicted by one of skill in the art from a hydrophobicity plot, determined for example, from a Hopp/Woods hydrophilicity profile based on a sliding six-residue window, with buried G, S, and T residues and exposed H, Y, and W residues ignored. Mouse zcytor10 hydrophilic peptides include peptides comprising amino acid sequences selected from the group consisting of: (1) amino acid number 150 (Arg) to amino acid number 155 (Asp) of SEQ ID NO:2; (2) amino acid number 254 (Arg) to amino acid number 259 (Ala) of SEQ ID NO:2; (3) amino acid number 296 (Ala) to amino acid number 301 (Glu) of SEQ ID NO:2; (4) amino acid number 297 (Arg) to amino acid number 302 (Asp) of SEQ ID NO:2; and (5) amino acid number 310 (Lys) to amino acid number 315 (Glu) of SEQ ID NO:2. The corresponding zcytor10 hydrophilic peptides of SEQ ID NO:35 are also included with comparison of the above hydrophilic peptides SEQ ID NO:2 in reference to SEQ ID NO:35. In addition, conserved motifs, and variable regions between conserved motifs of mouse zcytor10 are suitable antigens. Antibodies generated from this immune response can be isolated and purified as described herein. Methods for preparing and isolating polyclonal and monoclonal antibodies are well known in the art. See, for example, Current Protocols in Immunology, Cooligan, et al. (eds.), National Institutes of Health, John Wiley and Sons, Inc., 1995; Sambrook et al., Molecular Cloning: A Laboratory Manual, Second Edition, Cold Spring Harbor, NY, 1989; and Hurrell, J. G. R., Ed., Monoclonal Hybridoma Antibodies: Techniques and Applications, CRC Press, Inc., Boca Raton, FL, 1982.

As would be evident to one of ordinary skill in the art, polyclonal antibodies can be generated from inoculating a variety of warm-blooded animals such as horses, cows, goats, sheep, dogs, chickens, rabbits, mice, and rats with a mouse zcytor10 polypeptide or a fragment thereof. The immunogenicity of a mouse zcytor10 polypeptide may be increased through the use of an adjuvant, such as alum (aluminum hydroxide) or Freund's complete or incomplete adjuvant. Polypeptides useful for immunization also include fusion polypeptides, such as fusions of mouse zcytor10 or a

portion thereof with an immunoglobulin polypeptide or with maltose binding protein. The polypeptide immunogen may be a full-length molecule or a portion thereof. If the polypeptide portion is "hapten-like", such portion may be advantageously joined or linked to a macromolecular carrier (such as keyhole limpet hemocyanin (KLH), bovine serum albumin (BSA) or tetanus toxoid) for immunization.

As used herein, the term "antibodies" includes polyclonal antibodies, affinity-purified polyclonal antibodies, monoclonal antibodies, and antigen-binding fragments, such as $F(ab')_2$ and Fab proteolytic fragments. Genetically engineered intact antibodies or fragments, such as chimeric antibodies, Fv fragments, single chain antibodies and the like, as well as synthetic antigen-binding peptides and polypeptides, are also included. Non-human antibodies may be humanized by grafting non-human CDRs onto human framework and constant regions, or by incorporating the entire non-human variable domains (optionally "cloaking" them with a human-like surface by replacement of exposed residues, wherein the result is a "veneered" antibody). In some instances, humanized antibodies may retain non-human residues within the human variable region framework domains to enhance proper binding characteristics. Through humanizing antibodies, biological half-life may be increased, and the potential for adverse immune reactions upon administration to humans is reduced. Moreover, human antibodies can be produced in transgenic, non-human animals that have been engineered to contain human immunoglobulin genes as disclosed in WIPO Publication WO 98/24893. It is preferred that the endogenous immunoglobulin genes in these animals be inactivated or eliminated, such as by homologous recombination.

Antibodies are considered to be specifically binding if: 1) they exhibit a threshold level of binding activity, and 2) they do not significantly cross-react with related polypeptide molecules. A threshold level of binding is determined if anti-mouse zcytor10 antibodies herein bind to a mouse zcytor10 polypeptide, peptide or epitope with an affinity at least 10-fold greater than the binding affinity to control (non-mouse zcytor10) polypeptide. It is preferred that the antibodies exhibit a binding affinity (K_a) of 10^6 M^{-1} or greater, preferably 10^7 M^{-1} or greater, more preferably 10^8 M^{-1} or greater, and most preferably 10^9 M^{-1} or greater. The binding affinity of an antibody

can be readily determined by one of ordinary skill in the art, for example, by Scatchard analysis (Scatchard, G., Ann. NY Acad. Sci. 51: 660-672, 1949).

Whether anti-mouse zcytor10 antibodies do not significantly cross-react with related polypeptide molecules is shown, for example, by the antibody detecting mouse zcytor10 polypeptide but not known related polypeptides using a standard Western blot analysis (Ausubel et al., ibid.). Examples of known related polypeptides are those disclosed in the prior art, such as known orthologs, and paralogs, and similar known members of a protein family, Screening can also be done using non-mouse mouse zcytor10, and mouse zcytor10 mutant polypeptides. Moreover, antibodies can be "screened against" known related polypeptides, to isolate a population that specifically binds to the mouse zcytor10 polypeptides. For example, antibodies raised to mouse zcytor10 are adsorbed to related polypeptides adhered to insoluble matrix; antibodies specific to mouse zcytor10 will flow through the matrix under the proper buffer conditions. Screening allows isolation of polyclonal and monoclonal antibodies non-crossreactive to known closely related polypeptides (Antibodies: A Laboratory Manual, Harlow and Lane (eds.), Cold Spring Harbor Laboratory Press, 1988; Current Protocols in Immunology, Cooligan, et al. (eds.), National Institutes of Health, John Wiley and Sons, Inc., 1995). Screening and isolation of specific antibodies is well known in the art. See, Fundamental Immunology, Paul (eds.), Raven Press, 1993; Getzoff et al., *Adv. in Immunol.* 43: 1-98, 1988; Monoclonal Antibodies: Principles and Practice, Goding, J.W. (eds.), Academic Press Ltd., 1996; Benjamin et al., Ann. Rev. Immunol. 2: 67-101, 1984. Specifically binding anti-mouse zcytor10 antibodies can be detected by a number of methods in the art, and disclosed below.

A variety of assays known to those skilled in the art can be utilized to detect antibodies which bind to mouse zcytor10 proteins or polypeptides. Exemplary assays are described in detail in Antibodies: A Laboratory Manual, Harlow and Lane (Eds.), Cold Spring Harbor Laboratory Press, 1988. Representative examples of such assays include: concurrent immunoelectrophoresis, radioimmunoassay, radioimmunoprecipitation, enzyme-linked immunosorbent assay (ELISA), dot blot or Western blot assay, inhibition or competition assay, and sandwich assay. In addition, antibodies can

be screened for binding to wild-type versus mutant mouse zcytor10 protein or polypeptide.

Alternative techniques for generating or selecting antibodies useful herein include *in vitro* exposure of lymphocytes to mouse zcytor10 protein or peptide, and selection of antibody display libraries in phage or similar vectors (for instance, through use of immobilized or labeled mouse zcytor10 protein or peptide). Genes encoding polypeptides having potential mouse zcytor10 polypeptide binding domains can be obtained by screening random peptide libraries displayed on phage (phage display) or on bacteria, such as *E. coli*. Nucleotide sequences encoding the polypeptides can be obtained in a number of ways, such as through random mutagenesis and random polynucleotide synthesis. These random peptide display libraries can be used to screen for peptides that interact with a known target that can be a protein or polypeptide, such as a ligand or receptor, a biological or synthetic macromolecule, or organic or inorganic substances. Techniques for creating and screening such random peptide display libraries are known in the art (Ladner et al., US Patent NO. 5,223,409; Ladner et al., US Patent NO. 4,946,778; Ladner et al., US Patent NO. 5,403,484 and Ladner et al., US Patent NO. 5,571,698) and random peptide display libraries and kits for screening such libraries are available commercially, for instance from Clontech (Palo Alto, CA), Invitrogen Inc. (San Diego, CA), New England Biolabs, Inc. (Beverly, MA) and Pharmacia LKB Biotechnology Inc. (Piscataway, NJ). Random peptide display libraries can be screened using the mouse zcytor10 sequences disclosed herein to identify proteins which bind to mouse zcytor10. These "binding polypeptides" which interact with mouse zcytor10 polypeptides can be used for tagging cells; for isolating paralog and ortholog polypeptides by affinity purification; they can be directly or indirectly conjugated to drugs, toxins, radionuclides and the like. These binding polypeptides can also be used in analytical methods such as for screening expression libraries and neutralizing activity, e.g., for blocking interaction between ligand and receptor, or viral binding to a receptor. The binding polypeptides can also be used for diagnostic assays for determining circulating levels of mouse zcytor10 polypeptides or zcytor10 orthologs, e.g. in human samples; for detecting or quantitating soluble mouse zcytor10 polypeptides as marker of underlying pathology or disease in a mouse model,

or in human samples expressing zcytor10 orthologs. These binding polypeptides can also act as zcytor10 "antagonists" to block zcytor10 binding and signal transduction *in vitro* and *in vivo*. These anti-mouse zcytor10-binding polypeptides would be useful for inhibiting zcytor10 activity or protein binding.

Antibodies to mouse zcytor10 may be used for tagging cells that express mouse zcytor10; for isolating mouse zcytor10 by affinity purification; for diagnostic assays for determining circulating levels of mouse zcytor10 polypeptides or zcytor10 orthologs, e.g. in human samples; for detecting or quantitating soluble zcytor10 polypeptides as marker of underlying pathology or disease in a mouse model, or in human samples expressing zcytor10 orthologs; in analytical methods employing FACS; for screening expression libraries; for generating anti-idiotypic antibodies; and as neutralizing antibodies or as antagonists to block zcytor10 activity *in vitro* and *in vivo*. Suitable direct tags or labels include radionuclides, enzymes, substrates, cofactors, inhibitors, fluorescent markers, chemiluminescent markers, magnetic particles and the like; indirect tags or labels may feature use of biotin-avidin or other complement/anti-complement pairs as intermediates. Antibodies herein may also be directly or indirectly conjugated to drugs, toxins, radionuclides and the like, and these conjugates used for *in vivo* diagnostic or therapeutic applications. Moreover, antibodies to mouse zcytor10 or fragments thereof may be used *in vitro* to detect denatured mouse zcytor10 or fragments thereof in assays, for example, Western Blots or other assays known in the art.

Antibodies to mouse zcytor10 are useful for tagging cells that express the receptor and assaying mouse zcytor10 expression levels, for affinity purification, within diagnostic assays for determining circulating levels of soluble receptor polypeptides, analytical methods employing fluorescence-activated cell sorting. Divalent antibodies may be used as agonists to mimic the effect of the mouse zcytor10 ligand.

Antibodies herein can also be directly or indirectly conjugated to drugs, toxins, radionuclides and the like, and these conjugates used for *in vivo* diagnostic, in murine models to study therapeutic applications, or in therapeutic applications. For instance, antibodies or binding polypeptides which recognize mouse zcytor10 of the

present invention can be used to identify or treat tissues or organs that express a corresponding anti-complementary molecule (i.e., a mouse zcytor10 receptor). More specifically, anti-mouse zcytor10 antibodies, or bioactive fragments or portions thereof, can be coupled to detectable or cytotoxic molecules and delivered to a mammal having cells, tissues or organs that express the zcytor10 molecule.

Suitable detectable molecules may be directly or indirectly attached to polypeptides that bind mouse zcytor10 ("binding polypeptides," including binding peptides disclosed above), antibodies, or bioactive fragments or portions thereof. Suitable detectable molecules include radionuclides, enzymes, substrates, cofactors, inhibitors, fluorescent markers, chemiluminescent markers, magnetic particles and the like. Suitable cytotoxic molecules may be directly or indirectly attached to the polypeptide or antibody, and include bacterial or plant toxins (for instance, diphtheria toxin, *Pseudomonas* exotoxin, ricin, abrin and the like), as well as therapeutic radionuclides, such as iodine-131, rhenium-188 or yttrium-90 (either directly attached to the polypeptide or antibody, or indirectly attached through means of a chelating moiety, for instance). Binding polypeptides or antibodies may also be conjugated to cytotoxic drugs, such as adriamycin. For indirect attachment of a detectable or cytotoxic molecule, the detectable or cytotoxic molecule can be conjugated with a member of a complementary/ anticomplementary pair, where the other member is bound to the binding polypeptide or antibody portion. For these purposes, biotin/streptavidin is an exemplary complementary/ anticomplementary pair.

In another embodiment, binding polypeptide-toxin fusion proteins or antibody-toxin fusion proteins can be used for targeted cell or tissue inhibition or ablation (for instance, to treat cancer cells or tissues). Alternatively, if the binding polypeptide has multiple functional domains (i.e., an activation domain or a ligand binding domain, plus a targeting domain), a fusion protein including only the targeting domain may be suitable for directing a detectable molecule, a cytotoxic molecule or a complementary molecule to a cell or tissue type of interest. In instances where the fusion protein including only a single domain includes a complementary molecule, the anti-complementary molecule can be conjugated to a detectable or cytotoxic molecule. Such domain-complementary molecule fusion proteins thus represent a generic

targeting vehicle for cell/tissue-specific delivery of generic anti-complementary-detectable/ cytotoxic molecule conjugates.

In another embodiment, mouse zcytor10 binding polypeptide-cytokine or antibody-cytokine fusion proteins can be used for enhancing *in vivo* killing of target tissues (for example, blood, lymphoid, colon, and bone marrow cancers), if the binding polypeptide-cytokine or anti-mouse zcytor10 antibody targets the hyperproliferative cell (See, generally, Hornick et al., Blood 89:4437-47, 1997). They described fusion proteins enable targeting of a cytokine to a desired site of action, thereby providing an elevated local concentration of cytokine. Suitable anti-mouse zcytor10 antibodies target an undesirable cell or tissue (i.e., a tumor or a leukemia), and the fused cytokine mediates improved target cell lysis by effector cells. Suitable cytokines for this purpose include interleukin 2 and granulocyte-macrophage colony-stimulating factor (GM-CSF), for instance.

Alternatively, mouse zcytor10 binding polypeptide or antibody fusion proteins described herein can be used for enhancing *in vivo* killing of target tissues by directly stimulating a zcytor10-modulated apoptotic pathway, resulting in cell death of hyperproliferative cells expressing zcytor10 or orthologous sequences that cross-react with the antibody or binding polypeptide, such as human zcytor10.

The bioactive binding polypeptide or antibody conjugates described herein can be delivered orally, intravenously, intraarterially or intraductally, or may be introduced locally at the intended site of action.

Polynucleotides encoding mouse zcytor10 polypeptides are useful within gene therapy applications where it is desired to increase or inhibit mouse zcytor10 activity. If a mammal has a mutated or absent zcytor10 gene, the zcytor10 gene can be introduced into the cells of the mammal. In one embodiment, a gene encoding a mouse zcytor10 polypeptide is introduced *in vivo* in a viral vector. Such vectors include an attenuated or defective DNA virus, such as, but not limited to, herpes simplex virus (HSV), papillomavirus, Epstein Barr virus (EBV), adenovirus, adeno-associated virus (AAV), and the like. Defective viruses, which entirely or almost entirely lack viral genes, are preferred. A defective virus is not infective after introduction into a cell. Use of defective viral vectors allows for administration to cells in a specific, localized

area, without concern that the vector can infect other cells. Examples of particular vectors include, but are not limited to, a defective herpes simplex virus 1 (HSV1) vector (Kaplitt et al., Molec. Cell. Neurosci. 2:320-30, 1991); an attenuated adenovirus vector, such as the vector described by Stratford-Perricaudet et al., J. Clin. Invest. 90:626-30, 1992; and a defective adeno-associated virus vector (Samulski et al., J. Virol. 61:3096-101, 1987; Samulski et al., J. Virol. 63:3822-8, 1989).

In another embodiment, a mouse zcytor10 gene can be introduced in a retroviral vector, e.g., as described in Anderson et al., U.S. Patent No. 5,399,346; Mann et al. Cell 33:153, 1983; Temin et al., U.S. Patent No. 4,650,764; Temin et al., U.S. Patent No. 4,980,289; Markowitz et al., J. Virol. 62:1120, 1988; Temin et al., U.S. Patent No. 5,124,263; International Patent Publication No. WO 95/07358, published March 16, 1995 by Dougherty et al.; and Kuo et al., Blood 82:845, 1993. Alternatively, the vector can be introduced by lipofection *in vivo* using liposomes. Synthetic cationic lipids can be used to prepare liposomes for *in vivo* transfection of a gene encoding a marker (Felgner et al., Proc. Natl. Acad. Sci. USA 84:7413-7, 1987; Mackey et al., Proc. Natl. Acad. Sci. USA 85:8027-31, 1988). The use of lipofection to introduce exogenous genes into specific organs *in vivo* has certain practical advantages. Molecular targeting of liposomes to specific cells represents one area of benefit. For instance, directing transfection to particular cell types would be particularly advantageous in a tissue with cellular heterogeneity, such as the pancreas, liver, kidney, and brain. Lipids may be chemically coupled to other molecules for the purpose of targeting. Targeted peptides (e.g., hormones or neurotransmitters), proteins such as antibodies, or non-peptide molecules can be coupled to liposomes chemically.

It is possible to remove the target cells from the body; to introduce the vector as a naked DNA plasmid; and then to re-implant the transformed cells into the body. Naked DNA vectors for gene therapy can be introduced into the desired host cells by methods known in the art, e.g., transfection, electroporation, microinjection, transduction, cell fusion, DEAE dextran, calcium phosphate precipitation, use of a gene gun or use of a DNA vector transporter. See, e.g., Wu et al., J. Biol. Chem. 267:963-7, 1992; Wu et al., J. Biol. Chem. 263:14621-4, 1988. Mouse models employing

mzctyor10 can be used to study the application, safety, efficacy, and perfect such gene therapy techniques and applications discussed in the paragraphs above.

Antisense methodology can be used to inhibit mouse zcytor10 gene transcription, such as to inhibit cell proliferation *in vivo*. Polynucleotides that are complementary to a segment of a mouse zcytor10-encoding polynucleotide (e.g., a polynucleotide as set froth in SEQ ID NO:1) are designed to bind to mouse zcytor10-encoding mRNA and to inhibit translation of such mRNA. Such antisense polynucleotides are used to inhibit expression of mouse zcytor10 polypeptide-encoding genes in cell culture or in a mouse model for use in studying human disease, and studying the application, safety, efficacy, and perfection of antisense therapy methods.

In addition, as a cell surface molecule, mouse zcytor10 polypeptide can be used as a target to introduce gene therapy into a cell. This application would be particularly appropriate for introducing therapeutic genes into cells in which mouse zcytor10 is normally expressed, for example, lymphoid tissue and PBLs, or cancer cells which may express mouse zcytor10 polypeptide. For example, viral gene therapy, such as described above, can be targeted to specific cell types in which express a cellular receptor, such as mouse zcytor10 polypeptide, rather than the viral receptor. Antibodies, or other molecules that recognize mouse zcytor10 molecules on the target cell's surface can be used to direct the virus to infect and administer gene therapeutic material to that target cell. See, Woo, S.L.C, Nature Biotech. 14:1538, 1996; Wickham, T.J. et al, Nature Biotech. 14:1570-1573, 1996; Douglas, J.T et al., Nature Biotech. 14:1574-1578, 1996; Rihova, B., Crit. Rev. Biotechnol. 17:149-169, 1997; and Vile, R.G. et al., Mol. Med. Today 4:84-92, 1998. For example, a bispecific antibody containing a virus-neutralizing Fab fragment coupled to a mouse zcytor10-specific antibody can be used to direct the virus to cells expressing the mouse zcytor10 receptor and allow efficient entry of the virus containing a genetic element into the cells. See, for example, Wickham, T.J., et al., J. Virol. 71:7663-7669, 1997; and Wickham, T.J., et al., J. Virol. 70:6831-6838, 1996. Mouse models employing mzctyor10 can be used to study the application, safety, efficacy, and perfect such gene therapy techniques and applications discussed above.

The present invention also provides reagents which will find use in diagnostic applications. For example, the mouse zcytor10 gene, a probe comprising mouse zcytor10 DNA or RNA or a subsequence thereof can be used to determine the location of the murine zcytor10 gene on a mouse chromosome, of if a mouse zcytor10 ortholog gene is present on a human chromosome, or if a mutation has occurred. Detectable chromosomal aberrations at the mouse zcytor10 gene locus include, but are not limited to, aneuploidy, gene copy number changes, insertions, deletions, restriction site changes and rearrangements. Such aberrations can be detected using polynucleotides of the present invention by employing molecular genetic techniques, such as restriction fragment length polymorphism (RFLP) analysis, fluorescence *in situ* hybridization methods, short tandem repeat (STR) analysis employing PCR techniques, and other genetic linkage analysis techniques known in the art (Sambrook et al., ibid.; Ausubel et. al., ibid.; Marian, Chest 108:255-65, 1995).

Radiation hybrid mapping is a somatic cell genetic technique developed for constructing high-resolution, contiguous maps of mammalian chromosomes (Cox et al., Science 250:245-50, 1990). Partial or full knowledge of a gene's sequence allows one to design PCR primers suitable for use with chromosomal radiation hybrid mapping panels. Radiation hybrid mapping panels are commercially available which cover the entire human genome, such as the Stanford G3 RH Panel and the GeneBridge 4 RH Panel (Research Genetics, Inc., Huntsville, AL). These panels enable rapid, PCR-based chromosomal localizations and ordering of genes, sequence-tagged sites (STSs), and other nonpolymorphic and polymorphic markers within a region of interest. This includes establishing directly proportional physical distances between newly discovered genes of interest and previously mapped markers. The precise knowledge of a gene's position can be useful for a number of purposes, including: 1) determining if a sequence is part of an existing contig and obtaining additional surrounding genetic sequences in various forms, such as YACs, BACs or cDNA clones; 2) providing a possible candidate gene for an inheritable disease which shows linkage to the same chromosomal region; and 3) cross-referencing model organisms, such as mouse, which may aid in determining what function a particular human zcytor10 ortholog gene might have.

Sequence tagged sites (STSs) can also be used independently for chromosomal localization. An STS is a DNA sequence that is unique in the human genome and can be used as a reference point for a particular chromosome or region of a chromosome. An STS is defined by a pair of oligonucleotide primers that are used in a polymerase chain reaction to specifically detect this site in the presence of all other genomic sequences. Since STSs are based solely on DNA sequence they can be completely described within an electronic database, for example, Database of Sequence Tagged Sites (dbSTS), GenBank, (National Center for Biological Information, National Institutes of Health, Bethesda, MD http://www.ncbi.nlm.nih.gov), and can be searched with a gene sequence of interest for the mapping data contained within these short genomic landmark STS sequences.

Moreover, mouse zcytor10 mutant and transgenic mice, can be used as a mouse model for human genetic diseases. Over-expression or under-expression of the native mzyctor10 locus may result in a murine phenotype that corresponds to a human heritable disease state. Similarly, defects or mutations in the mouse zcytor10 locus itself may result in a murine phenotype that corresponds to a human heritable disease state. Molecules of the present invention, such as the polypeptides, antagonists, agonists, polynucleotides and antibodies of the present invention would aid in the detection, diagnosis prevention, and treatment associated with a mouse zcytor10 genetic defect, corresponding to a defect in the human ortholog, and aid in the understanding of human disease.

Mice engineered to express the mouse zcytor10 gene, referred to as "transgenic mice," and mice that exhibit a complete absence of mouse zcytor10 gene function, referred to as "knockout mice," can also be generated (Snouwaert et al., Science 257:1083, 1992; Lowell et al., Nature 366:740-42, 1993; Capecchi, M.R., Science 244: 1288-1292, 1989; Palmiter, R.D. et al. Annu Rev Genet. 20: 465-499, 1986). For example, transgenic mice that over-express mouse zcytor10, either ubiquitously or under a tissue-specific or tissue-restricted promoter can be used to ask whether over-expression causes a phenotype. For example, over-expression of a wild-type mouse zcytor10 polypeptide, polypeptide fragment or a mutant thereof may alter normal cellular processes, resulting in a phenotype that identifies a tissue in which

mouse zcytor10 expression is functionally relevant and may indicate a therapeutic target for the mouse zcytor10, its agonists or antagonists. For example, a preferred transgenic mouse to engineer is one that expresses a "dominant-negative" phenotype, such as one that over-expresses the mouse zcytor10 extracellular cytokine binding domain (approximately amino acids 15 (Cys) to 230 (Pro) of SEQ ID NO:2, or amino acids 17 (Ala) to 232 (Pro) of SEQ ID NO:35) with a transmembrane domain attached (for example, approximately amino acids 15 (Cys) to 251 (Leu) of SEQ ID NO:2, or amino acids 17 (Ala) to 254 (Leu) of SEQ ID NO:35) if the mouse zcytor10 transmembrane were used). Moreover, such over-expression may result in a phenotype that shows similarity with human diseases. Similarly, knockout mouse zcytor10 mice can be used to determine where mouse zcytor10 is absolutely required *in vivo*. The phenotype of knockout mice is predictive of the *in vivo* effects of that a mouse zcytor10 antagonist, such as those described herein, may have. These mice may be employed to study the mouse zcytor10 gene and the protein encoded thereby in an *in vivo* system, and can be used as *in vivo* models for corresponding human diseases. Moreover, transgenic mice expression of mouse zcytor10 antisense polynucleotides or ribozymes directed against mouse zcytor10, described herein, can be used analogously to transgenic mice described above.

The invention is further illustrated by the following non-limiting examples.

EXAMPLES

Example 1

Identification of mouse zcytor10

Using an EST Sequence and screening a murine cDNA Library to Obtain Full-length mouse zcytor10

A. Summary

Scanning of a translated DNA database resulted in identification of an mouse expressed sequence tag (EST) sequence found to be a partial sequence for a member of the Class I Cytokine Receptor family. After hybridization screening a

murine cDNA library the full-length clone was identified and sequenced. This Class I Cytokine Receptor was designated mouse zcytor10.

B. Using EST Sequence probe to screen a murine cDNA Library

A 433 base pair probe was generated using primers ZC14603 (SEQ ID NO:5), and ZC14606 (SEQ ID NO:6), 0.5 ng cDNA encoding the original EST (EST631772) as a template in a PCR reaction. KlenTaq™ polymerase (Clontech) and buffer were used. The PCR reaction conditions were as follows: followed by 30 cycles of 95°C for 30 sec., 60° C for 15 sec., 72°C for 1 min.; followed by 72°C for 10 min.; followed by a 10°C soak. A sample of the PCR reaction product was run on a 1.5% agarose gel. A band of the expected size of approximately 400 bp was seen. The PCR product was purified using a chromaspin 400 column (Clontech). The purified product was radioactively labeled with ^{32}P-dCTP using Rediprime II™ (Amersham), a random prime labeling system, according to the manufacturer's specifications. The probe was then purified using a Nuc-Trap™ column (Stratagene, La Jolla, CA) according to the manufacturer's instructions.

One million clones from a Mouse 17 day embryo cDNA library (Clontech), constructed in LambdaTriplEx™ (Clontech), were screened. Library plating and screening was performed according to the manufacturer's instructions. Twenty ng of ^{32}P-dCTP-labeled probe, described above, was used to probe twenty filters representing one million phage clones. The filters were washed in 0.25XSSC, 0.1% SDS, at 60°C. Two positive phages were obtained from the screening. The phagmid from the phage were isolated by *in vitro* excision according to the direction of the library vendor. Four phagmid clones, designated pSLMR10-1, -2, -3, -4, were submitted to sequencing.

Confirmation of the full-length sequence was made by sequence analyses from one clone, pSLMR10-2, which was sequenced using the following primers: ZC14603 (SEQ ID NO:5), ZC14606 (SEQ ID NO:6), ZC694 (SEQ ID NO:7), ZC8938 (SEQ ID NO:8), ZC16549 (SEQ ID NO:9), and ZC16550 (SEQ ID NO:10). The insert was 1455 bp, and was full-length.

Example 2

Mouse Zcytor10 Receptor Tissue Distribution

Northern blot analysis was performed using Mouse Multiple Tissue Northern™ Blots (Mouse MTN and Mouse Embryo MTN) (Clontech). The full length mouse probe was generated by PCR from the mouse cDNA plasmid as template and oligos ZC17,213 (SEQ ID NO:11) and ZC17,314 (SEQ ID NO:12) as primers. PCR conditions were as follows: 35 cycles at 95°C for 1 minute, 55 for 1minute, 72 for 2 minutes; 72°C for 10 minutes; 4°C overnight A sample of the PCR reaction product was run on 1% low melting point agarose gel (Boehringer Mannheim, Indianapolis, IN). A band of the expected size of 1.9 kb was seen. The 1.9 kb PCR fragment, was gel purified using a commercially available kit (Qiaquick Gel Extraction Kit™; Qiagen, Valencia CA) and radioactively labeled with ^{32}P-dCTP using Prime It II™ (Stratagene), a random prime labeling system, according to the manufacturer's specifications. The probe was purified using a Nuc-Trap™ column (Stratagene) according to the manufacturer's instructions. ExpressHyb™ (Clontech) solution was used for prehybridization and as a hybridizing solution for the Northern blots. Hybridization took place for 2 hours at 65°C using 1-2 x 10^6 cpm/ml of labeled probe. The blots were then washed 4 times for 15 minutes in 2X SSC/1% SDS at 25°C, followed by three30 minutes washes in 0.1X SSC/0.1% SDS at 50°C.A transcript of approximately 1.35 kb was detected in heart, spleen, lung, liver and testis. A weak band was observed in mouse seven-day embryo.

Example 3

Rat Zcytor10 Sequence

A rat EST was identified as an ortholog of the murine zcytor10 receptor. The EST contained the zcytor10 transmembrane domain as well as the entire intracellular region through the stop codon. Oligos were designed, ZC 24,055 (SEQ ID NO:13) and ZC 23,711 (SEQ ID NO:14). A rat kidney cDNA library (Clontech) was screened using the above oligos in a PCR reaction with the following conditions: 35 cycles at 95degrees for 1 minute, 55 degrees for 1 minute, and 72 degrees for 1 minute; 72 degrees for 10 minutes; 4 degrees overnight. A sample of the PCR reaction product

was run on 1% low melting point agarose gel (Boehringer Mannheim). A band of the expected size of approximately 300 bp was seen. The 300 bp PCR fragment, was gel purified using a commercially available kit (Qiaquick Gel Extraction Kit™; Qiagen). Sequencing confirmed the PCR fragment to be the intracellular and transmembrane portion of the rat zcytor10. The rat cDNA sequence is shown in SEQ ID NO:15 and corresponding amino acid sequence shown in SEQ ID NO:16. The transmembrane, intracellular domain, class I cytokine motifs and the like correlate with those shown as shown in the mouse zcytor10 sequence (SEQ ID NO:2).

Example 4

Rat Zcytor10 Tissue Distribution

Rat Multiple Tissue Northern Blots (Origene, Rockville, MD) were probed to determine the tissue distribution of rat zcytor10 expression. An approximately 250 bp PCR derived probe was amplified using rat cDNA (Example 3) as a template and oligonucleotide ZC23711 (SEQ ID NO:14) and ZC23712 (SEQ ID NO:17) as primers. PCR reaction conditions were as follows: 30 cycles of 90°C for 1 min., 55°C for 1 min., 72°C for 1.5 min.; 72°C for 10 min.; 4°C overnight. A sample of the PCR reaction product was run on 1% low melting point agarose gel (Boehringer Mannheim). A band of the expected size of 250 bp was seen. The 250 bp PCR fragment, was gel purified using a commercially available kit (Qiaquick Gel Extraction Kit™; Qiagen). The probe was radioactively labeled using the MULTIPRIME™ labeling kit (Amersham) according to the manufacturer's instructions. The probe was purified using a NUCTRAP™ push column (Stratagene). EXPRESSHYB™ (Clontech) solution was used for prehybridization and as a hybridizing solution for the Northern blots. Hybridization took place overnight at 60°C using about 10^6 cpm/ml of labeled probe. The blots were then washed one time in 6X SSC and 0.1% SDS at room temperature, followed by 5 washes in 6X SSC and 0.1% SDS at 60°C. A transcript of approximately 1.3 kb was seen in stomach, small intestine, lung, testis, skin, brain, kidney, spleen, thymus, and liver. A larger transcript of about 3.0 kb was seen in skeletal muscle and all the above tissues with the exception of spleen. For testis there

was an additional transcript at 1.0 kb and there was also a larger 6.0 kb transcript seen for thymus and testis.

Example 5

Construction of Mouse MPL-zcytor10 Polypeptide Chimera: MPL Extracellular and TM Domain Fused to the zcytor10 Intracellular Signaling Domain

The extracellular and transmembrane domains of the MPL receptor were isolated from a plasmid containing the MPL receptor (Souyri et al., Cell 63:1137-1147, 1990) (designated PHZ1/MPL plasmid) using PCR with primers ZC17,212 (SEQ ID NO:18) and ZC17,313 (SEQ ID NO:19). The PCR reaction conditions were as follows: 95°C for 1 min.; 35 cycles at 95°C for 1 min., 45°C for 1 min., 72°C for 2 min.; followed by 72°C at 10 min.; then a 10°C soak. The PCR product was run on a 1% low melting point agarose (Boerhinger Mannheim, Indianapolis, IN) and the approximately 1.5 kb MPL receptor fragment isolated using Qiaquick™ gel extraction kit (Qiagen) as per manufacturer's instructions.

The intracellular domain of zcytor10 was isolated from a plasmid containing zcytor10 receptor cDNA using PCR with primers ZC17,315 (SEQ ID NO:20) and ZC17,314 (SEQ ID NO:21). The polynucleotide sequence corresponding to the zcytor10 receptor intracellular domain coding sequence (amino acids 252 (Arg) to 357 (Leu) of SEQ ID NO:2) is shown in SEQ ID NO:1. The reaction conditions were as per above. The PCR product was run on a 1% low melting point agarose (Boerhinger Mannheim) and the approximately 350 bp zcytor10 fragment isolated using Qiaquick gel extraction kit (Qiagen) as per manufacturer's instructions.

Each of the isolated fragments described above were mixed at a 1:1 volumetric ratio and used in a PCR reaction using ZC17,212 (SEQ ID NO:18) and ZC17,314 (SEQ ID NO:21) to create the MPL-zcytor10 chimera. The reaction conditions were as follows: 95°C for 1 min.; 35 cycles at 95°C for 1 min., 55°C for 1 min., 72°C for 2 min.; followed by 72°C at 10 min.; then a 10°C soak. The entire PCR product was run on a 1% low melting point agarose (Boehringer Mannheim) and the approximately 1.9 kb MPL-zcytor10 chimera fragment isolated using Qiaquick gel extraction kit (Qiagen) as per manufacturer's instructions. The MPL-zcytor10 chimera

fragment was digested with EcoRI (BRL) and XbaI (Boerhinger Mannheim) as per manufacturer's instructions. The entire digest was run on a 1% low melting point agarose (Boehringer Mannheim) and the cleaved MPL-zcytor10 chimera isolated using Qiaquick™ gel extraction kit (Qiagen) as per manufacturer's instructions. The resultant cleaved MPL-zcytor10 chimera was inserted into an expression vector as described below.

Recipient expression vector pZP-5Z was digested with EcoRI (BRL) and HindIII (BRL) as per manufacturer's instructions, and gel purified as described above. This vector fragment was combined with the EcoRI and XbaI cleaved MPL-zcytor10 chimera isolated above and a XbaI/HindIII linker fragment in a ligation reaction. The ligation was run using T4 Ligase (BRL), at 15°C overnight. A sample of the ligation was electroporated in to DH10B ElectroMAX™ electrocompetent *E. coli* cells (25μF, 200Ω, 2.3V). Transformants were plated on LB+Ampicillin plates and single colonies screened by PCR to check for the MPL-zcytor10 chimera using ZC17,212 (SEQ ID NO:18) and ZC 17,314 (SEQ ID NO:21) using the PCR conditions as described above. Confirmation of the MPL-zcytor10 chimera sequence was made by sequence analyses. The insert was approximately 1.9 kb, and was full-length. The plasmid DNA was designated pZP-5Z/MPL-zcytor10.

Example 6

MPL-zcytor10 Chimera Based Proliferation in BAF3 Assay Using Alamar Blue

A. Construction of BaF3 Cells Expressing MPL-zcytor10 Chimera

BaF3, an interleukin-3 (IL-3) dependent pre-lymphoid cell line derived from murine bone marrow (Palacios and Steinmetz, Cell 41: 727-734, 1985; Mathey-Prevot et al., Mol. Cell. Biol. 6: 4133-4135, 1986), was maintained in complete media (RPMI medium (JRH Bioscience Inc., Lenexa, KS) supplemented with 10% heat-inactivated fetal calf serum, 2ng/ml murine IL-3 (mIL-3) (R & D, Minneapolis, MN), 2mM L-glutaMax-1™ (Gibco BRL), 1 mM Sodium Pyruvate (Gibco BRL), and PSN antibiotics (GIBCO BRL)). Prior to electroporation, pZP-5Z/MPL-zcytor10 DNA (Example 4) was prepared and purified using a Qiagen Maxi Prep kit (Qiagen) as per manufacturer's instructions. BaF3 cells for electroporation were washed once in RPMI

media and then resuspended in RPMI media at a cell density of 10^7 cells/ml. One ml of resuspended BaF3 cells was mixed with 30 µg of pZP-5Z/MPL-zcytor10 plasmid DNA (Example 5) and transferred to separate disposable electroporation chambers (GIBCO BRL). Following a 15 minute incubation at room temperature the cells were given two serial shocks (800 lFad/300 V.; 1180 lFad/300 V.) delivered by an electroporation apparatus (CELL-PORATOR™: GIBCO BRL). After a 5 minute recovery time, the electroporated cells were transferred to 50 ml of complete media and placed in an incubator for 15-24 hours (37°C, 5% CO_2). The cells were then spun down and resuspended in 50 ml of complete media containing Geneticin™ (Gibco) selection (500 µg/ml G418) in a T-162 flask to isolate the G418-resistant pool. Pools of the transfected BaF3 cells, hereinafter called BaF3/MPL-zcytor10 cells, were assayed for signaling capability as described below.

B. Testing the Signaling Capability of the BaF3/MPL-zcytor10Cells Using an Alamar Blue Proliferation Assay

BaF3/MPL-zcytor10 cells (Example 6A) were spun down and washed in the complete media, described above, but without mIL-3 (hereinafter referred to as "mIL-3 free media"). The cells were spun and washed 3 times to ensure the removal of the mIL-3. Cells were then counted in a hemacytometer. Cells were plated in a 96-well format at 5000 cells per well in a volume of 100 µl per well using the mIL-3 free media.

Proliferation of the BaF3/MPL-zcytor10 cells was assessed using thrombopoietin (TPO) diluted with mIL-3 free media to 1000 ng/ml, 500 ng/ml, 250 ng/ml, 125 ng/ml, 62 ng/ml, 30 ng/ml, 15 ng/ml, 7.5 ng/ml concentrations. 100 µl of the diluted TPO was added to the BaF3/MPL-zcytor10 cells. The total assay volume is 200 µl. Negative controls were run in parallel using mIL-3 free media only, without the addition of TPO. The assay plates were incubated at 37°C, 5% CO_2 for 3 days at which time Alamar Blue (Accumed, Chicago, IL) was added at 20µl/well. Alamar Blue gives a fluourometric readout based on number of live cells, and is thus a direct measurement of cell proliferation in comparison to a negative control. Plates were again incubated at 37°C, 5% CO_2 for 24 hours. Plates were read on the Fmax™ plate

reader (Molecular Devices Sunnyvale, CA) using the SoftMax™ Pro program, at wavelengths 544 (Excitation) and 590 (Emmission).

Results showed no proliferation of the Baf3/Mpl-zcytor10 chimera cell line in response to TPO suggesting that the intracellular portion of the zcytor10 molecule is incapable of signaling as a homodimer.

Example 7

Construction of Mouse Zcytor10-mpl Polypeptide Chimera: Zcytor10 Extracellular Domain Fused to the Mpl Intracellular Signaling Domain and TM Domain

The extracellular domains of the zcytor10 receptor were isolated from a plasmid containing the zcytor10 receptor using PCR with primers ZC17,213 (SEQ ID NO:11) and ZC17,204 (SEQ ID NO:22). The reaction conditions were as follows: 95°C for 1 min.; 35 cycles at 95°C for 1 min., 45°C for 1 min., 72°C for 2 min.; followed by 72°C at 10 min.; then a 10°C soak. The PCR product was run on a 1% low melting point agarose (Boerhinger Mannheim, Indianapolis, IN) and the approximately 800 bp zcytor10 receptor fragment isolated using Qiaquick™ gel extraction kit (Qiagen) as per manufacturer's instructions.

The intracellular and transmembrane domains of MPL were isolated from a plasmid containing MPL receptor cDNA (PHZ1/MPL plasmid) (Example 5) using PCR with primers ZC17,205 (SEQ ID NO:23) and ZC17,206 (SEQ ID NO:24). The reaction conditions were run as per above. The PCR product was run on a 1% low melting point agarose (Boerhinger Mannheim) and the approximately 450 bp MPL fragment isolated using Qiaquick gel extraction kit (Qiagen) as per manufacturer's instructions.

Each of the isolated fragments described above were mixed at a 1:1 volumetric ratio and used in a PCR reaction using ZC17,213 (SEQ ID NO:11) and ZC17,206 (SEQ ID NO:24) to create a Zcytor10-mpl chimera. The reaction conditions were as follows: 95°C for 1 min.; 35 cycles at 95°C for 1 min., 55°C for 1 min., 72°C for 2 min.; followed by 72°C at 10 min.; then a 10°C soak. The entire PCR product was run on a 1% low melting point agarose (Boehringer Mannheim) and an approximately 1.2 kb Zcytor10-mpl chimera fragment isolated using Qiaquick gel extraction kit

(Qiagen) as per manufacturer's instructions. The Zcytor10-mpl chimera fragment was digested with EcoRI (BRL) and XbaI (Boerhinger Mannheim) as per manufacturer's instructions. The entire digest was run on a 1% low melting point agarose (Boehringer Mannheim) and the cleaved Zcytor10-mpl chimera isolated using Qiaquick™ gel extraction kit (Qiagen) as per manufacturer's instructions. The resultant cleaved Zcytor10-mpl chimera was inserted into an expression vector as described below.

Recipient expression vector pZP-5Z was digested with EcoRI (BRL) and HindIII (BRL) as per manufacturer's instructions, and gel purified as described above. This vector fragment was combined with the EcoRI and XbaI cleaved Zcytor10-mpl chimera isolated above and a XbaI/HindIII linker fragment in a ligation reaction. The ligation was run using T4 Ligase (BRL), at 15°C overnight. A sample of the ligation was electroporated in to DH10B ElectroMAX™ electrocompetent *E. coli* cells (25μF, 200Ω, 2.3V). Transformants were plated on LB+Ampicillin plates and single colonies screened by PCR to check for the Zcytor10-mpl chimera using ZC17,213 (SEQ ID NO:11) and ZC 17,206 (SEQ ID NO:24) using the PCR conditions as described above.

Confirmation of the Zcytor10-mpl chimera sequence was made by sequence analyses. The insert was approximately 1.2 kb, and was full-length.

Example 8

Construction of Expression Vector Expressing Full-length zcytor10

The entire zcytor10 receptor was isolated from a plasmid containing zcytor10 receptor cDNA using PCR with primers ZC17,213 (SEQ ID NO:11) and ZC17,314 (SEQ ID NO:21). The reaction conditions were as follows: 95°C for 1 min; 35 cycles at 95°C for 1 min, 55°C for 1 min, 72°C for 2 min; followed by 72°C at 10 min; then a 10°C soak. The PCR product was run on a 1% low melting point agarose (Boerhinger Mannheim) and the approximately 1.1 kb zcytor10 cDNA isolated using Qiaquick™ gel extraction kit (Qiagen) as per manufacturer's instructions.

The purified zcytor10 cDNA was digested with EcoRI (BRL) and XbaI (Boehringer Mannheim) as per manufacturer's instructions. The entire digest was run on a 1% low melting point agarose (Boerhinger Mannheim) and purified the cleaved zcytor10 fragment using Qiaquick gel extraction kit (Qiagen) as per manufacturer's

instructions. The resultant cleaved zcytor10 was inserted into an expression vector as described below.

Recipient expression vector pZP-5N was digested with EcoRI (BRL) and XbaI (Boerhinger Mannheim) as per manufacturer's instructions, and gel purified as described above. This vector fragment was combined with the EcoRI and XbaI cleaved zcytor10 fragment isolated above in a ligation reaction. The ligation was run using T4 Ligase (BRL), at 15°C overnight. A sample of the ligation was electroporated in to DH10B electroMAX™ electrocompetent *E. coli* cells (25μF, 200Ω, 2.3V). Transformants were plated on LB+Ampicillin plates and single colonies screened by PCR to check for the zcytor10 sequence using ZC17,213 (SEQ ID NO:11) and ZC17,314 (SEQ ID NO:21) using the PCR conditions as described above. Confirmation of the full-length zcytor10 sequence was made by sequence analyses. The insert was approximately 1.1kb, and was full-length.

Example 9

Construction of cells to assess zcytor10 based proliferation in BAF3 assay using Alamar Blue

A. Construction of BaF3 Cells Expressing Zcytor10-MPL receptor

BaF3 cells expressing the Zcytor10-MPL receptor were constructed as per Example 6A, using 30μg of the zcytor10 expression vector, described in Example 7. The BaF3 cells expressing the pZP-5Z/zcytor10 receptor plasmid were designated as BaF3/Zcytor10-mpl. These cells were used to screen for a zcytor10 activity as described below in Examples 10 and 18.

B. Construction of BaF3 Cells Expressing zcytor10 receptor

BaF3 cells expressing the full-length zcytor10 receptor were constructed as per Example 6A, using 30μg of the zcytor10 expression vector, described in Example 8. The BaF3 cells expressing the pZP-5Z/zcytor10 receptor plasmid were designated as BaF3/zcytor10. These cells were used to screen for a zcytor10 activity as described below in Examples 10 and 18.

Example 10

Screening for zcytor10 activity using BaF3/zcytor10-MPL cells and Baf3/zcytor10 cells using an Alamar Blue Proliferation Assay

Baf3/zcytor10-mpl chimera cells and Baf3/zcytor10 cells (Example 9) were spun down and washed independently mIL-3 free media (Example 6). The cells were spun and washed 3 times to ensure the removal of the mIL-3. Cells were then counted in a hemacytometer. Cells were plated in a 96-well format at 5000 cells per well in a volume of 100 µl per well using the mIL-3 free media.

To try and identify a source for the zcytor10 ligand, approximately 124 conditioned media samples from a variety of cell lines were screened. 100 µl of each conditioned media sample was added to the BaF3/MPL-zcytor10 chimera cells as well as the Baf3/zcytor10 cells. The total assay volume was 200 µl. All known cytokines were also screened at a concentration of 250 ng/ml on both cell lines. Negative controls were run in parallel using mIL-3 free media only. Mouse IL-3 at a concentration of 250 pg/ml was used as a positive control. The assay plates were incubated at 37°C, 5% CO_2 for 3 days at which time Alamar Blue (Accumed, Chicago, IL) was added at 20µl/well. Alamar Blue gives a fluourometric readout based on number of live cells, and is thus a direct measurement of cell proliferation in comparison to a negative control. Plates were again incubated at 37°C, 5% CO_2 for 24 hours. Plates were read on the Fmax™ plate reader (Molecular Devices Sunnyvale, CA) using the SoftMax™ Pro program, at wavelengths 544 (Excitation) and 590 (Emission).

Results showed no proliferation of on either the Baf3/zcytor10-mpl chimera cell line or the Baf3/zcytor10 cell line in response to conditioned media samples or the known ligands. This result suggested that the zcytor10 receptor may not signal as a homodimer. The actual receptor-signaling complex may require another receptor subunit not present in BaF3 cells. See example 18 and Example19 below.

Example 11

Construction of Mammalian Expression Vectors That Express zcytor10 Soluble Receptors: zcytor10CEE, zcytor10CFLG, zcytor10CHIS and zcytor10-Fc4

A. Construction of zcytor10 Mammalian Expression Vector containing zcytor10CEE, zcytor10CFLG and zcytor10CHIS

An expression vector is prepared for the expression of the soluble, extracellular domain of the zcytor10 polypeptide, pC4zcytor10CEE, wherein the construct is designed to express a zcytor10 polypeptide comprised of the predicted initiating methionine and truncated adjacent to the predicted transmembrane domain, and with a C-terminal Glu-Glu tag (SEQ ID NO:25).

A zcytor10 DNA fragment comprising the zcytor10 extracellular cytokine binding domain (amino acid 15 (Cys) to 230 (Pro) of SEQ ID NO:2) is created using PCR, and purified for example, as described in Example 7. The excised DNA is subcloned into a plasmid expression vector that has a signal peptide, e.g., the native zcytor10 signal peptide, and attaches a Glu-Glu tag (SEQ ID NO:25) to the C-terminus of the zcytor10 polypeptide-encoding polynucleotide sequence. Such an expression vector mammalian expression vector contains an expression cassette having a mammalian promoter, multiple restriction sites for insertion of coding sequences, a stop codon and a mammalian terminator. The plasmid can also have an *E. coli* origin of replication, a mammalian selectable marker expression unit having an SV40 promoter, enhancer and origin of replication, a DHFR gene and the SV40 terminator.

Restriction digested zcytor10 insert and previously digested vector are ligated using standard molecular biological techniques, and electroporated into competent cells such as DH10B competent cells (GIBCO BRL, Gaithersburg, MD) according to manufacturer's direction and plated onto LB plates containing 50 mg/ml ampicillin, and incubated overnight. Colonies are screened by restriction analysis of DNA prepared from individual colonies. The insert sequence of positive clones is verified by sequence analysis. A large scale plasmid preparation is done using a QIAGEN® Maxi prep kit (Qiagen) according to manufacturer's instructions.

The same process is used to prepare the zcytor10 soluble receptors with a C-terminal his tag, composed of 6 His residues in a row; and a C-terminal flag (SEQ

ID NO:26) tag, zcytor10CFLAG. To construct these constructs, the aforementioned vector has either the HIS or the FLAG® tag in place of the glu-glu tag (SEQ ID NO:25).

B. Mammalian Expression Construction of Soluble zcytor10 receptor zcytor10-Fc4

An expression plasmid containing all or part of a polynucleotide encoding zcytor10 is constructed via homologous recombination. A fragment of zcytor10 cDNA was isolated using PCR that includes the polynucleotide sequence from extracellular domain of the zcytor10 receptor. Primers used in PCR for the production of the zcytor10 fragment are from 5' to 3' end: (1) about 40 bp of the vector flanking sequence (5' of the insert) and about17 bp corresponding to the 5' end of the zcytor10 extracellular domain; and (2) about 40 bp of the 5' end of the Fc4 polynucleotide sequence (SEQ ID NO:27) and about 17 bp corresponding to the 3' end of the zcytor10 extracellular domain. The fragment of Fc-4 for fusion with the zcytor10 is generated by PCR in a similar fashion. The two primers used in the production of the Fc4 fragment include: (1) a 5' primer consisting of about 40 bp of sequence from the 3' end of zcytor10 extracellular domain and about 17 bp of the 5' end of Fc4 (SEQ ID NO:27); and (2) a 3' primer consisting of about 40 bp of vector sequence (3' of the insert) and about 17 bp of the 3' end of Fc4 (SEQ ID NO:27). PCR amplification of the each of the reactions described above is then performed using conditions standard in the art.

An exemplary expression vector is derived from the plasmid pCZR199 (deposited at the American Type Culture Collection, 10801 University Boulevard, Manassas, VA 20110-2209, designated No. 98668), that is cut with SmaI (BRL). The expression vector was derived from the plasmid pCZR199, and is a mammalian expression vector containing an expression cassette having the CMV immediate early promoter, a consensus intron from the variable region of mouse immunoglobulin heavy chain locus, multiple restriction sites for insertion of coding sequences, a stop codon and a human growth hormone terminator. The expression vector also has an *E. coli* origin of replication, a mammalian selectable marker expression unit having an SV40 promoter, enhancer and origin of replication, a DHFR gene and the SV40 terminator.

The expression vector used was constructed from pCZR199 by the replacement of the metallothionein promoter with the CMV immediate early promoter.

Competent yeast cells (*S. cerevisiae*) are combined with approximately 1 μg each of the zcytor10 and Fc4 inserts, and 100 ng of SmaI (BRL) digested expression vector and electroporated. The yeast/DNA mixtures are electropulsed at, for example, 0.75 kV (5 kV/cm), "infinite" ohms, 25 μF. To each cuvette is added 600 μl of 1.2 M sorbitol and the yeast was plated in aliquots onto URA-D plates and incubated at 30°C.

After about 48 hours, the Ura+ yeast transformants from a single plate are picked, DNA isolated, and transformed into electrocompetent *E. coli* cells (e.g., DH10B, GibcoBRL), and plated using standard procedures. Individual clones harboring the correct expression construct for zcytor10-Fc4 are identified by restriction digest to verify the presence of the zcytor10-Fc4 insert and to confirm that the various DNA sequences have been joined correctly to one another. The insert of positive clones is subjected to sequence analysis. Larger scale plasmid DNA is isolated using the Qiagen Maxi kit (Qiagen) according to manufacturer's instructions.

Example 12

Transfection And Expression Of Zcytor10 Soluble Receptor Polypeptides

BHK 570 cells (ATCC No. CRL-10314), DG-44 CHO, or other mammalian cells are plated at about $1.2X10^6$ cells/well (6-well plate) in 800 μl of appropriate serum free (SF) media (e.g., DMEM, Gibco/BRL High Glucose) (Gibco BRL, Gaithersburg, MD). The cells are transfected with expression plasmids containing zcytor10CEE, zcytor10CFLG, zcytor10CHIS or zcytor10-Fc4 (Example 11), using Lipofectin™ (Gibco BRL), in serum free (SF) media according to manufacturer's instruction. Single clones expressing the soluble receptors are isolated, screened and grown up in cell culture media, and purified using standard techniques.

Example 13

Expression of zcytor10 soluble receptor in *E. coli*

A. Construction of expression vector pCZR225 that expresses huzcytor10/MBP-6H fusion polypeptide

An expression plasmid containing a polynucleotide encoding a zcytor10 soluble receptor fused C-terminally to maltose binding protein (MBP) is constructed via homologous recombination. The fusion polypeptide contains an N-terminal approximately 388 amino acid MBP portion fused to the zcytor10 soluble receptor (amino acid 15 (Cys) to amino acid 230 (Pro) of SEQ ID NO:2). A fragment of zcytor10 cDNA (SEQ ID NO:1) is isolated using PCR as described herein. Two primers are used in the production of the zcytor10 fragment in a standard PCR reaction: (1) one containing about 40 bp of the vector flanking sequence and about 25 bp corresponding to the amino terminus of the zcytor10, and (2) another containing about 40 bp of the 3' end corresponding to the flanking vector sequence and about 25 bp corresponding to the carboxyl terminus of the zcytor10. Two µl of the 100 µl PCR reaction is run on a 1.0% agarose gel with 1 x TBE buffer for analysis, and the expected approximately fragment is seen. The remaining PCR reaction is combined with the second PCR tube and precipitated with 400 µl of absolute ethanol. The precipitated DNA used for recombining into the SmaI cut recipient vector pTAP98 to produce the construct encoding the MBP-zcytor10 fusion, as described below.

Plasmid pTAP98 is derived from the plasmids pRS316 and pMAL-c2. The plasmid pRS316 is a *Saccharomyces cerevisiae* shuttle vector (Hieter P. and Sikorski, R., Genetics 122:19-27, 1989). pMAL-C2 (NEB) is an *E. coli* expression plasmid. It carries the tac promoter driving *MalE* (gene encoding MBP) followed by a His tag, a thrombin cleavage site, a cloning site, and the *rrnB* terminator. The vector pTAP98 is constructed using yeast homologous recombination. 100ng of EcoR1 cut pMAL-c2 is recombined with 1µg PvuI cut pRS316, 1µg linker, and 1µg ScaI/EcoR1 cut pRS316 are combined in a PCR reaction. PCR products are concentrated via 100% ethanol precipitation.

Competent yeast cells (*S. cerevisiae*) are combined with about 10 µl of a mixture containing approximately 1 µg of the zcytor10 receptor PCR product above, and 100 ng of SmaI digested pTAP98 vector, and electroporated using standard methods and plated onto URA-D plates and incubated at 30°C.

After about 48 hours, the Ura+ yeast transformants from a single plate are picked, DNA isolated, and transformed into electrocompetent *E. coli* cells (e.g., MC1061, Casadaban et. al. J. Mol. Biol. 138, 179-207), and plated on MM/CA +AMP 100 mg/L plates (Pryor and Leiting, Protein Expression and Pruification 10:309-319, 1997).using standard procedures. Cells are grown in MM/CA with 100 µg/ml Ampicillin for two hours, shaking, at 37°C. 1ml of the culture is induced with 1mM IPTG. 2-4 hours later the 250 µl of each culture is mixed with 250 µl acid washed glass beads and 250 µl Thorner buffer with 5% βME and dye (8M urea, 100 mM Tris pH7.0, 10% glycerol, 2mM EDTA, 5% SDS). Samples are vortexed for one minute and heated to 65°C for 10 minutes. 20 µl are loaded per lane on a 4%-12% PAGE gel (NOVEX). Gels are run in 1XMES buffer. The positive clones are designated pCZR225 and subjected to sequence analysis.

One microliter of sequencing DNA is used to transform strain BL21. The cells are electropulsed at 2.0 kV, 25 µF and 400 ohms. Following electroporation, 0.6 ml MM/CA with 100 mg/L Ampicillin. Cells are grown in MM/CA and induced with ITPG as described above., The positive clones are used to grow up for protein purification of the huzcytor10/MBP-6H fusion protein using standard techniques.

Example 14

Zcytor10 Soluble Receptor Polyclonal Antibodies

Polyclonal antibodies are prepared by immunizing female New Zealand white rabbits with the purified huzcytor10/MBP-6H polypeptide (Example 13), or the purified recombinant zcytor10CEE soluble receptor (Example 11). The rabbits are each given an initial intraperitoneal (IP) injection of 200 mg of purified protein in Complete Freund's Adjuvant (Pierce, Rockford, IL) followed by booster IP injections of 100 mg purified protein in Incomplete Freund's Adjuvant every three weeks. Seven to ten days

after the administration of the third booster injection, the animals are bled and the serum is collected. The rabbits are then boosted and bled every three weeks.

The zcytor10-specific polyclonal antibodies are affinity purified from the rabbit serum using an CNBr-SEPHAROSE 4B protein column (Pharmacia LKB) that is prepared using about 10 mg of the purified huzcytor10/MBP-6H polypeptide per gram CNBr-SEPHAROSE, followed by 20X dialysis in PBS overnight. Zcytor10-specific antibodies are characterized by an ELISA titer check using 1 mg/ml of the appropriate protein antigen as an antibody target. The lower limit of detection (LLD) of the rabbit anti-zcytor10 affinity purified antibodies is determined using standard methods.

Example 15

Zcytor10 receptor Monoclonal Antibodies

Zcytor10 soluble receptor Monoclonal antibodies are prepared by immunizing male BalbC mice (Harlan Sprague Dawley, Indianapolis, IN) with the purified recombinant soluble zcytor10 proteins described herein. The mice are each given an initial intraperitoneal (IP) injection of 20 mg of purified protein in Complete Freund's Adjuvant (Pierce, Rockford, IL) followed by booster IP injections of 10 mg purified protein in Incomplete Freund's Adjuvant every two weeks. Seven to ten days after the administration of the third booster injection, the animals are bled and the serum is collected, and antibody titer assessed.

Splenocytes are harvested from high-titer mice and fused to murine SP2/0 myeloma cells using PEG 1500 (Boerhinger Mannheim, UK) in two separate fusion procedures using a 4:1 fusion ratio of splenocytes to myeloma cells (Antibodies: A Laboratory Manual, E. Harlow and D. Lane, Cold Spring Harbor Press). Following 10 days growth post-fusion, specific antibody-producing hybridomas are identified by ELISA using purified recombinant zcytor10 soluble receptor protein (Example 6C) as an antibody target and by FACS using Baf3 cells expressing the zcytor10 sequence (Example 8) as an antibody target. The resulting hybridomas positive by both methods are cloned three times by limiting dilution.

Example 16

Assessing Zcytor10 Receptor Heterodimerization using ORIGEN assay

Soluble zcytor10 receptor zcytor10CFLAG (Example 11), or gp130 (Hibi, M. et al., Cell 63:1149-1157, 1990) are biotinylated by reaction with a five-fold molar excess of sulfo-NHS-LC-Biotin (Pierce, Inc., Rockford, IL) according to the manufacturer's protocol. Soluble zcytor10 receptor and another soluble receptor subunit, for example, soluble IL-7Rα (sIL-7Rα) or IL-2 receptor-γ (sIL-2Rγ) (R&D Systems, Minneapolis, MN), or soluble zalpha11 receptor (IL-21R; commonly owned US Pat. Application No. 09/404,641) are labeled with a five fold molar excess of Ru-BPY-NHS (Igen, Inc., Gaithersburg, MD) according to manufacturer's protocol. The biotinylated and Ru-BPY-NHS-labeled forms of the soluble zcytor10 receptor can be respectively designated Bio-zcytor10 receptor and Ru-zcytor10; the biotinylated and Ru-BPY-NHS-labeled forms of the other soluble receptor subunit can be similarly designated. Assays can be carried out using conditioned media from cells expressing a ligand that binds zcytor10 heterodimeric receptors, or using purified ligands. Preferred ligands are those that can bind class 1 heterodimeric cytokine receptors such as, IL-2, IL-4, IL-7, IL-9, IL-15, zalpha11 Ligand (IL-21) (commonly owned US Pat. Application No. 09/522,217), TSLP (Levine, SD et al., ibid.; Isaksen, DE et al., ibid.; Ray, RJ et al., ibid.; Friend, SL et al., ibid.).

For initial receptor binding characterization a panel of cytokines or conditioned medium are tested to determine whether they can mediate homodimerization of zcytor10 receptor and if they can mediate the heterodimerization of zcytor10 receptor with the soluble receptor subunits described above. To do this, 50 µl of conditioned media or TBS-B containing purified cytokine, is combined with 50 µl of TBS-B (20 mM Tris, 150 mM NaCl, 1 mg/ml BSA, pH 7.2) containing e.g., 400 ng/ml of Ru-zcytor10 receptor and Bio-zcytor10, or 400 ng/ml of Ru-zcytor10 receptor and e.g., Bio-gp130, or 400 ng/ml of e.g., Ru-IL2Rγ and Bio-zcytor10. Following incubation for one hour at room temperature, 30 µg of streptavidin coated, 2.8 mm magnetic beads (Dynal, Inc., Oslo, Norway) are added and the reaction incubated an additional hour at room temperature. 200 µl ORIGEN assay buffer (Igen, Inc.,

Gaithersburg, MD) is then added and the extent of receptor association measured using an M8 ORIGEN analyzer (Igen, Inc.).

Example 17

Construct for generating a zcytor10 receptor Heterodimer

A vector expressing a secreted human zcytor10 heterodimer is constructed. In this construct, the extracellular cytokine-binding domain of zcytor10 is fused to the heavy chain of IgG gamma 1 (IgGγ1) (SEQ ID NO:28 and SEQ ID NO:29), while the extracellular portion of the heteromeric cytokine receptor subunit (E.g., an IL-2 receptor component (IL-2Rα, IL-2Rβ, IL-2Rγ), an IL-4/IL-13 receptor family receptor components (IL-4Rα, IL-13Rα, IL-13Rα'), interleukin receptor subunits (e.g., IL-15 Rα, IL-7Rα, IL-9Rα); or zalpha11 receptor (IL-21R)) is fused to a human kappa light chain (human κ light chain) (SEQ ID NO:30 and SEQ ID NO:31).

A. Construction of IgG gamma 1 and human κ light chain fusion vectors

The heavy chain of IgGγ1 is cloned into the Zem229R mammalian expression vector (ATCC deposit No. 69447) such that any desired cytokine receptor extracellular domain having a 5' EcoRI and 3' NheI site can be cloned in resulting in an N-terminal extracellular domain-C-terminal IgGγ1 fusion. The IgGγ1 fragment used in this construct is made by using PCR to isolate the IgGγ1 sequence from a Clontech hFetal Liver cDNA library as a template. PCR products are purified using methods described herein and digested with MluI and EcoRI (Boerhinger-Mannheim), ethanol precipitated and ligated with oligos ZC11,440 (SEQ ID NO:32) and ZC11,441 (SEQ ID NO:33), which comprise an MluI/EcoRI linker, into Zem229R previously digested with and EcoRI using standard molecular biology techniques disclosed herein.

The human κ light chain (SEQ ID NO:30 and SEQ ID NO:31) is cloned in the Zem228R mammalian expression vector (ATCC deposit No. 69446) such that any desired cytokine receptor extracellular domain having a 5' EcoRI site and a 3' KpnI site can be cloned in resulting in a N-terminal cytokine extracellular domain-C-terminal human κ light chain fusion. As a KpnI site is located within the human κ light chain sequence (cleaved by the KpnI enzyme after nucleotide 62 in SEQ ID NO:30), a special primer is designed to clone the 3' end of the desired extracellular domain of a cytokine

receptor into this KpnI site: The primer is designed so that the resulting PCR product contains the desired cytokine receptor extracellular domain with a segment of the human κ light chain up to the KpnI site (SEQ ID NO:36). This primer preferably comprises a portion of at least 10 nucleotides of the 3' end of the desired cytokine receptor extracellular domain fused in frame 5' to SEQ ID NO:36. The human κ light chain fragment used in this construct is made by using PCR to isolate the human κ light chain sequence from the same Clontech human Fetal Liver cDNA library used above. PCR products are purified using methods described herein and digested with MluI and EcoRI (Boerhinger-Mannheim), ethanol precipitated and ligated with the MluI/EcoRI linker described above, into Zem228R previously digested with and EcoRI using standard molecular biology techniques disclosed herein.

B. Insertion of zcytor10 receptor or heterodimeric subunit extracellular domains into fusion vector constructs

Using the construction vectors above, a construct having zcytor10 fused to IgGγ1 is made. This construction is done by PCRing the extracellular cytokine-binding domain of zcytor10 receptor (amino acids 15 (Cys) to 230 (Pro) of SEQ ID NO:2) from a kidney cDNA library (Clontech) using standard methods (E.g., Example 7), and oligos that provide EcoRI and NheI restriction sites. The resulting PCR product is digested with EcoRI and NheI, gel purified, as described herein, and ligated into a previously EcoRI and NheI digested and band-purified Zem229R/IgGγ1 described above. The resulting vector is sequenced to confirm that the zcytor10/IgG gamma 1 fusion (zcytor10/Ch1 IgG) is correct.

A separate construct having a heterodimeric cytokine receptor subunit extracellular domain fused to κ light is also constructed as above. The IL-2Rγ/human κ light chain construction is performed as above by PCRing from, e.g., a lymphocyte cDNA library (Clontech) using standard methods, and oligos that provide EcoRI and KpnI restriction sites. The resulting PCR product is digested with EcoRI and KpnI and then ligating this product into a previously EcoRI and KpnI digested and band-purified Zem228R/human κ light chain vector described above. The resulting vector is

sequenced to confirm that the cytokine receptor subunit/human κ light chain fusion is correct.

D. Co-expression of the zcytor10 and heterodimeric cytokine receptor subunit extracellular domain

Approximately 15 μg of each of vectors above, are co-transfected into mammalian cells, e.g., BHK-570 cells (ATCC No. CRL-10314) using LipofectaminePlus™ reagent (Gibco/BRL), as per manufacturer's instructions. The transfected cells are selected for 10 days in DMEM + 5%FBS (Gibco/BRL) containing 1 μM of methotrexate (MTX) (Sigma, St. Louis, MO) and 0.5 mg/ml G418 (Gibco/BRL) for 10 days. The resulting pool of transfectants is selected again in 10 μm of MTX and 0.5 mg/ml G418 for 10 days.

The resulting pool of doubly selected cells is used to generate protein. Three Factories (Nunc, Denmark) of this pool are used to generate 10 L of serum free conditioned medium. This conditioned media is passed over a 1 ml protein-A column and eluted in about 10, 750 microliter fractions. The fractions having the highest protein concentration are pooled and dialyzed (10 kD MW cutoff) against PBS. Finally the dialyzed material is submitted for amino acid analysis (AAA) using routine methods.

Example 18

Determination of receptor subunits that heterodimerize or multimerize with zcytor10 receptor.

Using standard methods described herein, The BaF3/MPL-zcytor10 chimera cells (Example 6) are transfected with an additional heterodimeric cytokine receptor subunit serve as a bioassay cell line to measure signal transduction response of heterodimeric zcytor10 receptor complexes to the luciferase reporter in the presence of TPO (Example 6). In the presence of TPO, the BaF3/MPL-zcytor10 cells do not signal, suggesting that zcytor10 receptor must heterodimerize to signal. Transfection of the BaF3/MPL-zcytor10 cell line with and additional MPL-class I cytokine receptor fusion that signals in the presence of the TPO ligand, determines which heterodimeric cytokine

receptor subunits are required for zcytor10 receptor signaling. Use of MPL-receptor fusions for this purpose alleviates the requirement for the presence of a natural ligand for the zcytor10 receptor.

MPL-class I cytokine receptor fusions are made as per Example 5 using the extracellular domain and transmembrane domains of the MPL receptor and the intracellular signaling domain of the desired class I cytokine receptor. The BaF3/MPL-zcytor10 bioassay cell line co-transfected with an individual MPL-class I cytokine receptor fusions as per Example 6 to form a BaF3/MPL-zcytor10/MPL-class I cytokine receptor cell line. Receptor complexes include but are not limited to zcytor10 receptor in combination with an MPL-cytokine receptor fusion comprising one or more of the IL-2 receptor components (IL-2Rα, IL-2Rβ, IL-2Rγ), zcytor10 receptor with one or more of the IL-4/IL-13 receptor family receptor components (IL-4Rα, IL-13Rα, IL-13Rα'), as well as other Interleukin receptors (e.g., IL-15 Rα, IL-7Rα, IL-9Rα, IL-21R (Zalpha11 receptor)). Each independent receptor complex cell line is then assayed in the presence of TPO (example 6) and proliferation measured using routine methods (e.g., Alamar Blue assay as described in Example 6). The BaF3/MPL-zcytor10 bioassay cell line serves as a control for the background luciferase activity, and is thus used as a baseline to compare signaling by the various receptor complex combinations. In addition, a BaF3/MPL-class I cytokine receptor cell line can be constructed to control for MPL-class I cytokine receptor homodimerization effects for those class I cytokine receptors known to signal upon homodimerization. The TPO in the presence of the correct receptor complex, is expected to increase proliferation of the BaF3/MPL-zcytor10/MPL-class I cytokine receptor cell line approximately 5 fold over background or greater in the presence of TPO.

Example 19

Reconstitution of zcytor10 receptor *in vitro*

To identify components involved in the zcytor10-signaling complex, receptor reconstitution studies are performed as follows. BHK 570 cells (ATCC No. CRL-10314) transfected, using standard methods described herein, with a luciferase reporter mammalian expression vector plasmid serve as a bioassay cell line to measure

signal transduction response from a transfected zcytor10 receptor complex to the luciferase reporter in the presence of zcytor10 Ligand. BHK cells do not endogenously express the zcytor10 receptor. An exemplary luciferase reporter mammalian expression vector is the KZ134 plasmid which was constructed with complementary oligonucleotides ZC12,749 (SEQ ID NO:37) and ZC12,748 (SEQ ID NO:38) that contain STAT transcription factor binding elements from 4 genes. A modified c-fos Sis inducible element (m67SIE, or hSIE) (Sadowski, H. et al., Science 261:1739-1744, 1993), the p21 SIE1 from the p21 WAF1 gene (Chin, Y. et al., Science 272:719-722, 1996), the mammary gland response element of the β-casein gene (Schmitt-Ney, M. et al., Mol. Cell. Biol. 11:3745-3755, 1991), and a STAT inducible element of the Fcg RI gene, (Seidel, H. et al., Proc. Natl. Acad. Sci. 92:3041-3045, 1995). These oligonucleotides contain Asp718-XhoI compatible ends and were ligated, using standard methods, into a recipient firefly luciferase reporter vector with a c-fos promoter (Poulsen, L.K. et al., J. Biol. Chem. 273:6229-6232, 1998) digested with the same enzymes and containing a neomycin selectable marker. The KZ134 plasmid is used to stably transfect BHK, or BaF3 cells, using standard transfection and selection methods, to make a BHK/KZ134 or BaF3/KZ134 cell line respectively.

The bioassay cell line is transfected with zcytor10 receptor alone, or co-transfected with zcytor10 receptor along with one of a variety of other known receptor subunits. Receptor complexes include but are not limited to zcytor10 receptor only, various combinations of zcytor10 receptor with one or more of the IL-2 receptor components (IL-2Rα, IL-2Rβ, IL-2Rγ), zcytor10 receptor with one or more of the IL-4/IL-13 receptor family receptor components (IL-4Rα, IL-13Rα, IL-13Rα'), as well as other Interleukin receptors (e.g., IL-15 Rα, IL-7Rα, IL-9Rα, IL-21R (zalpha11)). Each independent receptor complex cell line is then assayed in the presence of cytokine-conditioned media or purified cytokines and luciferase activity measured using routine methods. The untransfected bioassay cell line serves as a control for the background luciferase activity, and is thus used as a baseline to compare signaling by the various receptor complex combinations. The conditioned medium or cytokine that binds the zyctor10 receptor in the presence of the correct receptor complex, is expected to give a luciferase readout of approximately 5 fold over background or greater.

As an alternative, a similar assay can be performed wherein the Baf3/zcytor10-mpl and Baf3/zcytor10 (Example 10) cell lines are co-transfected as described above and proliferation measured.

From the foregoing, it will be appreciated that, although specific embodiments of the invention have been described herein for purposes of illustration, various modifications may be made without deviating from the spirit and scope of the invention. Accordingly, the invention is not limited except as by the appended claims.

CLAIMS

What is claimed is:

1. An isolated polynucleotide that encodes a polypeptide comprising a sequence of amino acid residues that is at least 90% identical to an amino acid sequence selected from the group of:

(a) the amino acid sequence as shown in SEQ ID NO:2 from amino acid number 15 (Cys), to amino acid number 230 (Pro);

(b) the amino acid sequence as shown in SEQ ID NO:35 from amino acid number 17 (Ala), to amino acid number 232 (Pro);

(c) the amino acid sequence as shown in SEQ ID NO:2 from amino acid number 25 (Gly), to amino acid number 230 (Pro);

(d) the amino acid sequence as shown in SEQ ID NO:2 from amino acid number 15 (Cys), to amino acid number 251 (Leu);

(e) the amino acid sequence as shown in SEQ ID NO:35 from amino acid number 17 (Ala), to amino acid number 253 (Leu);

(f) the amino acid sequence as shown in SEQ ID NO:2 from amino acid number 252 (Arg), to amino acid number 357 (Leu);

(g) the amino acid sequence as shown in SEQ ID NO:2 from amino acid number 15 (Cys), to amino acid number 357 (Leu); and

(h) the amino acid sequence as shown in SEQ ID NO:35 from amino acid number 17 (Ala), to amino acid number 359 (Leu); and

(i) the amino acid sequence as shown in SEQ ID NO:2 from amino acid number 1 (Met) to amino acid number 357 (Leu)

(j) the amino acid sequence as shown in SEQ ID NO:35 from amino acid number 1 (Met) to amino acid number 359 (Leu).

2. An isolated polynucleotide according to claim 1, wherein the polypeptide comprises a sequence of amino acid residues that is selected from the group of:

(a) the amino acid sequence as shown in SEQ ID NO:2 from amino acid number 15 (Cys), to amino acid number 230 (Pro);

(b) the amino acid sequence as shown in SEQ ID NO:35 from amino acid number 17 (Ala), to amino acid number 232 (Pro);

(c) the amino acid sequence as shown in SEQ ID NO:2 from amino acid number 25 (Gly), to amino acid number 230 (Pro);

(d) the amino acid sequence as shown in SEQ ID NO:2 from amino acid number 15 (Cys), to amino acid number 251 (Leu);

(e) the amino acid sequence as shown in SEQ ID NO:35 from amino acid number 17 (Ala), to amino acid number 253 (Leu);

(f) the amino acid sequence as shown in SEQ ID NO:2 from amino acid number 252 (Arg), to amino acid number 357 (Leu);

(g) the amino acid sequence as shown in SEQ ID NO:2 from amino acid number 15 (Cys), to amino acid number 357 (Leu); and

(h) the amino acid sequence as shown in SEQ ID NO:35 from amino acid number 17 (Ala), to amino acid number 359 (Leu); and

(i) the amino acid sequence as shown in SEQ ID NO:2 from amino acid number 1 (Met) to amino acid number 357 (Leu)

(j) the amino acid sequence as shown in SEQ ID NO:35 from amino acid number 1 (Met) to amino acid number 359 (Leu).

3. An isolated polynucleotide according to claim 1, wherein the polypeptide further comprises a transmembrane domain consisting of residues 231 (Leu) to 251 (Leu) of SEQ ID NO:2.

4. An isolated polynucleotide according to claim 1 wherein the polypeptide further comprises an intracellular domain consisting of residues 252 (Arg) to 357 (Leu) of SEQ ID NO:2.

5. An expression vector comprising the following operably linked elements:

a transcription promoter;

a DNA segment encoding a zcytor10 polypeptide that is at least 90% identical to an amino acid sequence as shown in SEQ ID NO:2 from amino acid number 15 (Cys), or 25 (Gly) to amino acid number 357 (Leu); or is at least 90% identical to an amino acid sequence as shown in SEQ ID NO:35 from amino acid number 17 (Ala), to amino acid number 232 (Pro); and

a transcription terminator,

wherein the promoter is operably linked to the DNA segment, and the DNA segment is operably linked to the transcription terminator.

6. An expression vector according to claim 5, further comprising a secretory signal sequence operably linked to the DNA segment.

7. A cultured cell comprising an expression vector according to claim 5, wherein the cell expresses a polypeptide encoded by the DNA segment.

8. An expression vector according to claim 5, wherein the DNA segment encodes a zcytor10 polypeptide comprising an amino acid sequence as shown in SEQ ID NO:2 from amino acid number 15 (Cys), or 25 (Gly) to amino acid number 230 (Pro); or comprising an amino acid sequence as shown in SEQ ID NO:35 from amino acid number 17 (Ala), to amino acid number 232 (Pro); and

a transcription terminator,

wherein the promoter, DNA segment, and terminator are operably linked.

9. An expression vector according to claim 8, further comprising a secretory signal sequence operably linked to the DNA segment.

10. An expression vector according to claim 8, wherein the polypeptide further comprises a transmembrane domain consisting of residues 231 (Leu) to 251 (Leu) of SEQ ID NO:2.

11. An expression vector according to claim 8 wherein the polypeptide further comprises an intracellular domain consisting of residues 252 (Arg) to 357 (Leu) of SEQ ID NO:2.

12. A cultured cell into which has been introduced an expression vector according to claim 8, wherein the cell expresses a soluble receptor polypeptide encoded by the DNA segment.

13. A DNA construct encoding a fusion protein, the DNA construct comprising:.

a first DNA segment encoding a polypeptide that is at least 90% identical to a sequence of amino acid residues selected from the group of:

(a) the amino acid sequence of SEQ ID NO:2 from amino acid number 1 (Met), to amino acid number 14 (Gly);

(b) the amino acid sequence of SEQ ID NO:35 from amino acid number 1 (Met), to amino acid number 16 (Ala);

(c) the amino acid sequence of SEQ ID NO:2 from amino acid number 15 (Cys) to amino acid number 230 (Pro);

(d) the amino acid sequence of SEQ ID NO:35 from amino acid number 17 (Ala) to amino acid number 232 (Pro);

(e) the amino acid sequence of SEQ ID NO:2 from amino acid number 25 (Gly) to amino acid number 230 (Pro);

(f) the amino acid sequence of SEQ ID NO:2 from amino acid number 15 (Cys) to amino acid number 251 (Leu);

(g) the amino acid sequence of SEQ ID NO:2 from amino acid number 17 (Ala) to amino acid number 253 (Leu);

(h) the amino acid sequence of SEQ ID NO:2 from amino acid number 231 (Leu) to amino acid number 251 (Leu);

(h) the amino acid sequence of SEQ ID NO:2 from amino acid number 231 (Leu) to amino acid number 357 (Leu);

(j) the amino acid sequence of SEQ ID NO:2 from amino acid number 252 (Arg) to amino acid number 357 (Leu); and

(k) the amino acid sequence of SEQ ID NO:2 from amino acid number 15 (Cys), to amino acid number 357 (Leu); and

(l) the amino acid sequence of SEQ ID NO:2 from amino acid number 17 (Ala), to amino acid number 359 (Leu); and

at least one other DNA segment encoding an additional polypeptide,

wherein the first and other DNA segments are connected in-frame; and

wherein the first and other DNA segments encode the fusion protein.

14. An expression vector comprising the following operably linked elements:

a transcription promoter;

a DNA construct encoding a fusion protein according to claim 13; and

a transcription terminator,

wherein the promoter is operably linked to the DNA construct, and the DNA construct is operably linked to the transcription terminator.

15. A cultured cell comprising an expression vector according to claim 14, wherein the cell expresses a polypeptide encoded by the DNA construct.

16. A method of producing a fusion protein comprising:

culturing a cell according to claim 15; and

isolating the polypeptide produced by the cell.

17. An isolated polypeptide comprising a sequence of amino acid residues that is at least 90% identical to an amino acid sequence selected from the group of:

(a) the amino acid sequence as shown in SEQ ID NO:2 from amino acid number 15 (Cys), to amino acid number 230 (Pro);

(b) the amino acid sequence as shown in SEQ ID NO:35 from amino acid number 17 (Ala), to amino acid number 232 (Pro);

(c) the amino acid sequence as shown in SEQ ID NO:2 from amino acid number 25 (Gly), to amino acid number 230 (Pro);

(d) the amino acid sequence as shown in SEQ ID NO:2 from amino acid number 15 (Cys), to amino acid number 251 (Leu);

(e) the amino acid sequence as shown in SEQ ID NO:35 from amino acid number 17 (Ala), to amino acid number 253 (Leu);

(f) the amino acid sequence as shown in SEQ ID NO:2 from amino acid number 252 (Arg), to amino acid number 357 (Leu);

(g) the amino acid sequence as shown in SEQ ID NO:2 from amino acid number 15 (Cys), to amino acid number 357 (Leu); and

(h) the amino acid sequence as shown in SEQ ID NO:35 from amino acid number 17 (Ala), to amino acid number 359 (Leu); and

(i) the amino acid sequence as shown in SEQ ID NO:2 from amino acid number 1 (Met) to amino acid number 357 (Leu)

(j) the amino acid sequence as shown in SEQ ID NO:35 from amino acid number 1 (Met) to amino acid number 359 (Leu).

18. An isolated polypeptide according to claim 17, wherein the sequence of amino acid residues is selected from the group of:

(a) the amino acid sequence as shown in SEQ ID NO:2 from amino acid number 15 (Cys), to amino acid number 230 (Pro);

(b) the amino acid sequence as shown in SEQ ID NO:35 from amino acid number 17 (Ala), to amino acid number 232 (Pro);

(c) the amino acid sequence as shown in SEQ ID NO:2 from amino acid number 25 (Gly), to amino acid number 230 (Pro);

(d) the amino acid sequence as shown in SEQ ID NO:2 from amino acid number 15 (Cys), to amino acid number 251 (Leu);

(e) the amino acid sequence as shown in SEQ ID NO:35 from amino acid number 17 (Ala), to amino acid number 253 (Leu);

(f) the amino acid sequence as shown in SEQ ID NO:2 from amino acid number 252 (Arg), to amino acid number 357 (Leu);

(g) the amino acid sequence as shown in SEQ ID NO:2 from amino acid number 15 (Cys), to amino acid number 357 (Leu); and

(h) the amino acid sequence as shown in SEQ ID NO:35 from amino acid number 17 (Ala), to amino acid number 359 (Leu); and

(i) the amino acid sequence as shown in SEQ ID NO:2 from amino acid number 1 (Met) to amino acid number 357 (Leu)

(j) the amino acid sequence as shown in SEQ ID NO:35 from amino acid number 1 (Met) to amino acid number 359 (Leu).

19. An isolated polypeptide according to claim 17, wherein the polypeptide molecule encodes motifs 1, 2, 3, 4, 5 and 6 spaced apart from N-terminus to C-terminus in a configuration M1-{32-35}-M2-{31-32}-M3-{14-15}-M4-{11}-M5-{22-24}-M6,

wherein M1 is "motif 1," a sequence of amino acids as shown in SEQ ID NO:43,

M2 is "motif 2," a sequence of amino acids as shown in SEQ ID NO:44,

M3 is "motif 3," a sequence of amino acids consisting of LKP,

M4 is "motif 4," a sequence of amino acids consisting of VTV,

M5 is "motif 5," a sequence of amino acids as shown in SEQ ID NO:45, and

M6 is "motif 6," a sequence of amino acids consisting of GLD, and

{#} denotes the number of amino acids between the motifs.

20. An isolated polypeptide according to claim 17, wherein the polypeptide further comprises a transmembrane domain consisting of residues 231 (Leu) to 251 (Leu) of SEQ ID NO:2.

21. An isolated polypeptide according to claim 17 wherein the polypeptide further comprises an intracellular domain consisting of residues 252 (Arg) to 357 (Leu) of SEQ ID NO:2.

22. A method of producing a zcytor10 polypeptide comprising:

culturing a cell according to claim 7; and

isolating the zcytor10 polypeptide produced by the cell.

23. An isolated polypeptide according to claim 17 comprising an amino acid segment selected from the group of:

(a) the amino acid sequence as shown in SEQ ID NO:2 from amino acid number 15 (Cys), or 25 (Gly) to amino acid number 230 (Pro);

(a) the amino acid sequence as shown in SEQ ID NO:35 from amino acid number 17 (Ala), to amino acid number 232 (Pro); and

(b) sequences that are at least 90% identical to (a) or (b),

wherein the polypeptide is substantially free of transmembrane and intracellular domains ordinarily associated with hematopoietic receptors.

24. A method of producing a zcytor10 polypeptide comprising:

culturing a cell according to claim 12; and

isolating the zcytor10 polypeptide produced by the cell.

25. A method of producing an antibody to a zcytor10 polypeptide comprising:

inoculating an animal with a polypeptide selected from the group of:

(a) a polypeptide consisting of 9 to 343 amino acids, wherein the polypeptide is at least 90% identical to a contiguous sequence of amino acids in SEQ ID NO:2 from amino acid number 15 (Cys), to amino acid number 357 (Leu);

(b) a polypeptide consisting of 9 to 343 amino acids, wherein the polypeptide is at least 90% identical to a contiguous sequence of amino acids in SEQ ID NO:35 from amino acid number 17 (Ala), to amino acid number 359 (Leu);

(b) a polypeptide consisting of the amino acid sequence of SEQ ID NO:2 from amino acid number 25 (Gly), to amino acid number 230 (Pro);

(c) a polypeptide consisting of the amino acid sequence of SEQ ID NO:2 from amino acid number 114 (Lys) to amino acid number 121 (Val);

(d) a polypeptide consisting of the amino acid sequence of SEQ ID NO:2 from amino acid number 177 (Arg) to amino acid number 186 (Ala);

(e) a polypeptide consisting of the amino acid sequence of SEQ ID NO:2 from amino acid number 252 (Arg) to amino acid number 357 (Leu);

(f) a polypeptide consisting of the amino acid sequence of SEQ ID NO:2 from amino acid number 260 (Leu) to amino acid number 267 (Pro);

(g) a polypeptide consisting of the amino acid sequence of SEQ ID NO:2 from amino acid 298 (Thr) to amino acid number 302 (Asp);

(h) a polypeptide consisting of the amino acid sequence of SEQ ID NO:2 from amino acid 150 (Arg) to amino acid number 155 (Asp);

(i) a polypeptide consisting of the amino acid sequence of SEQ ID NO:2 from amino acid number 254 (Arg) to amino acid number 259 (Ala); and

(j) a polypeptide consisting of the amino acid sequence of SEQ ID NO:2 from amino acid number 296 (Ala) to amino acid number 301 (Glu);

(k) a polypeptide consisting of the amino acid sequence of SEQ ID NO:2 from amino acid 297 (Arg) to amino acid number 302 (Asp); and

(l) a polypeptide consisting of the amino acid sequence of SEQ ID NO:2 from amino acid number 310 (Lys) to amino acid number 315 (Glu)); and

wherein the polypeptide elicits an immune response in the animal to produce the antibody; and

isolating the antibody from the animal.

26. An antibody produced by the method of claim 25, which specifically binds to a zcytor10 polypeptide.

27. The antibody of claim 26, wherein the antibody is a monoclonal antibody.

28. An antibody which specifically binds to a polypeptide of claim 17.

29. A method of detecting, in a test sample, the presence of a modulator of zcytor10 protein activity, comprising:

culturing a cell into which has been introduced an expression vector according to claim 8, wherein the cell expresses the mouse zcytor10 protein encoded by the DNA segment in the presence and absence of a test sample; and

comparing levels of activity of mouse zcytor10 in the presence and absence of a test sample, by a biological or biochemical assay; and

determining from the comparison, the presence of modulator of zcytor10 activity in the test sample.

30. A method for detecting a zcytor10 receptor ligand within a test sample, comprising:

contacting a test sample with a polypeptide according to claim 17 comprising an amino acid sequence as shown in SEQ ID NO:2 from amino acid number 15 (Cys), or 25 (Gly) to amino acid number 230 (Pro); and

detecting the binding of the polypeptide to a ligand in the sample.

31. A method according to claim 30 wherein the polypeptide is membrane bound within a cultured cell, and the detecting step comprises measuring a biological response in the cultured cell.

32. A method according to claim 31 wherein the biological response is cell proliferation or activation of transcription of a reporter gene.

SEQUENCE LISTING

<110> ZymoGenetics, Inc.

<120> CYTOKINE RECEPTOR MOUSE ZCYTOR10

<130> 99-35PC

<150> US 09/309,861
<151> 1999-05-11

<160> 45

<170> FastSEQ for Windows Version 3.0

<210> 1
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tcccagcgcc ctagccaagg gtctgagtca gggga atg gct gcg atg gct ctt       233
                                      Met Ala Ala Met Ala Leu
                                       1               5

ctg gaa cgc gct gac gtc acg ggc tgc tcc cct gat ccc cgc cct gcc      281
Leu Glu Arg Ala Asp Val Thr Gly Cys Ser Pro Asp Pro Arg Pro Ala
            10                  15                  20

ccc gca ggt gat gtc aca gtc gtc tgc cat gac ctg gag acg gtg gag      329
Pro Ala Gly Asp Val Thr Val Val Cys His Asp Leu Glu Thr Val Glu
        25                  30                  35
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gtc acg tgg ggc tcg ggc ccc gac cac cac ggc gcc aac ttg agc ctg      377
Val Thr Trp Gly Ser Gly Pro Asp His His Gly Ala Asn Leu Ser Leu
 40                  45                  50

gag ttc cgt tat ggt act ggc gcc ctg caa ccc tgc ccg cga tat ttc      425
Glu Phe Arg Tyr Gly Thr Gly Ala Leu Gln Pro Cys Pro Arg Tyr Phe
 55                  60                  65                  70

ctg tcc ggc gct ggt gtc act tcc ggg tgc atc ctc ccc gcg gcg agg      473
Leu Ser Gly Ala Gly Val Thr Ser Gly Cys Ile Leu Pro Ala Ala Arg
                 75                  80                  85

gcg ggg ctg ctg gag ctg gca ctg cgc gac gga ggc ggg gcc atg gtg      521
Ala Gly Leu Leu Glu Leu Ala Leu Arg Asp Gly Gly Gly Ala Met Val
             90                  95                 100

ttt aag gct agg cag cgc gcg tcc gcc tgg ctg aag ccc cgc cca cct      569
Phe Lys Ala Arg Gln Arg Ala Ser Ala Trp Leu Lys Pro Arg Pro Pro
        105                 110                 115

tgg aat gtg acg ctg ctc tgg aca cca gac ggg gac gtg act gtc tcc      617
Trp Asn Val Thr Leu Leu Trp Thr Pro Asp Gly Asp Val Thr Val Ser
    120                 125                 130

tgg cct gcc cac tcc tac ctg ggc ctg gac tac gag gtg cag cac cgg      665
Trp Pro Ala His Ser Tyr Leu Gly Leu Asp Tyr Glu Val Gln His Arg
135                 140                 145                 150

gag agc aat gac gat gag gac gcc tgg cag acg acc tca ggg ccc tgc      713
Glu Ser Asn Asp Asp Glu Asp Ala Trp Gln Thr Thr Ser Gly Pro Cys
                155                 160                 165

tgt gac ttg aca gtg ggc ggg ctc gac ccc gcg cgc tgc tat gac ttc      761
Cys Asp Leu Thr Val Gly Gly Leu Asp Pro Ala Arg Cys Tyr Asp Phe
            170                 175                 180

cgg gtt cgg gcg tcg ccc cgg gcc gcg cac tat ggc ctg gag gcg cag      809
Arg Val Arg Ala Ser Pro Arg Ala Ala His Tyr Gly Leu Glu Ala Gln
        185                 190                 195

cct agc gag tgg aca gcg gtg aca agg ctt tcc ggg gca gca tcc gcg      857
Pro Ser Glu Trp Thr Ala Val Thr Arg Leu Ser Gly Ala Ala Ser Ala
    200                 205                 210
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Ala Ser Cys Thr Ala Ser Pro Ala Pro Ser Pro Ala Leu Ala Pro Pro
215                 220                 225                 230

ctc ctg ccc ctg ggc tgc ggc cta gca gcg ctg ctg aca ctg tcc ctg      953
Leu Leu Pro Leu Gly Cys Gly Leu Ala Ala Leu Leu Thr Leu Ser Leu
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ctc ctg gcc gcc ctg agg ctt cgc agg gtg aaa gat gcg ctg ctg ccc     1001
Leu Leu Ala Ala Leu Arg Leu Arg Arg Val Lys Asp Ala Leu Leu Pro
            250                 255                 260

tgc gtc cct gac ccc agc ggc tcc ttc cct gga ctc ttt gag aag cat     1049
Cys Val Pro Asp Pro Ser Gly Ser Phe Pro Gly Leu Phe Glu Lys His
        265                 270                 275

cac ggg aac ttc cag gcc tgg att gcg gac gcc cag gcc aca gcc ccg     1097
His Gly Asn Phe Gln Ala Trp Ile Ala Asp Ala Gln Ala Thr Ala Pro
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cca gcc agg acc gag gag gaa gat gac ctc atc cac ccc aag gct aag     1145
Pro Ala Arg Thr Glu Glu Glu Asp Asp Leu Ile His Pro Lys Ala Lys
295                 300                 305                 310

agg gtg gag ccc gag gac ggc acc tcc ctc tgc acc gtg cca agg cca     1193
Arg Val Glu Pro Glu Asp Gly Thr Ser Leu Cys Thr Val Pro Arg Pro
                315                 320                 325

ccc agc ttc gag cca agg ggg ccg gga ggc ggg gcc atg gtg tca gtg     1241
Pro Ser Phe Glu Pro Arg Gly Pro Gly Gly Gly Ala Met Val Ser Val
            330                 335                 340

ggc ggg gcc acg ttc atg gtg ggc gac agc ggc tac atg acc ctg         1286
Gly Gly Ala Thr Phe Met Val Gly Asp Ser Gly Tyr Met Thr Leu
        345                 350                 355

tgaccttgaa gtcactgcca gtctatactt caggctgagg tcacttcctg tctttaaata  1346
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        35                  40                  45
Gly Ala Asn Leu Ser Leu Glu Phe Arg Tyr Gly Thr Gly Ala Leu Gln
    50                  55                  60
Pro Cys Pro Arg Tyr Phe Leu Ser Gly Ala Gly Val Thr Ser Gly Cys
65                  70                  75                  80
Ile Leu Pro Ala Ala Arg Ala Gly Leu Leu Glu Leu Ala Leu Arg Asp
                85                  90                  95
Gly Gly Gly Ala Met Val Phe Lys Ala Arg Gln Arg Ala Ser Ala Trp
            100                 105                 110
Leu Lys Pro Arg Pro Pro Trp Asn Val Thr Leu Leu Trp Thr Pro Asp
        115                 120                 125
Gly Asp Val Thr Val Ser Trp Pro Ala His Ser Tyr Leu Gly Leu Asp
    130                 135                 140
Tyr Glu Val Gln His Arg Glu Ser Asn Asp Asp Glu Asp Ala Trp Gln
145                 150                 155                 160
Thr Thr Ser Gly Pro Cys Cys Asp Leu Thr Val Gly Gly Leu Asp Pro
                165                 170                 175
Ala Arg Cys Tyr Asp Phe Arg Val Arg Ala Ser Pro Arg Ala Ala His
            180                 185                 190
Tyr Gly Leu Glu Ala Gln Pro Ser Glu Trp Thr Ala Val Thr Arg Leu
        195                 200                 205
Ser Gly Ala Ala Ser Ala Ala Ser Cys Thr Ala Ser Pro Ala Pro Ser
    210                 215                 220
Pro Ala Leu Ala Pro Pro Leu Leu Pro Leu Gly Cys Gly Leu Ala Ala
225                 230                 235                 240
Leu Leu Thr Leu Ser Leu Leu Leu Ala Ala Leu Arg Leu Arg Arg Val
                245                 250                 255
Lys Asp Ala Leu Leu Pro Cys Val Pro Asp Pro Ser Gly Ser Phe Pro
            260                 265                 270
Gly Leu Phe Glu Lys His His Gly Asn Phe Gln Ala Trp Ile Ala Asp
        275                 280                 285
Ala Gln Ala Thr Ala Pro Pro Ala Arg Thr Glu Glu Glu Asp Asp Leu
    290                 295                 300
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Ile His Pro Lys Ala Lys Arg Val Glu Pro Glu Asp Gly Thr Ser Leu
305 310 315 320
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340 345 350
Gly Tyr Met Thr Leu
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<221> VARIANT
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<223> Xaa = Any Amino Acid

<400> 3
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1 5

<210> 4
<211> 1071
<212> DNA
<213> Artificial Sequence

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<221> misc_feature
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tggggnwsng gnccngayca ycayggngcn aayytnwsny tngarttymg ntayggnacn 180
ggngcnytnc arccntgycc nmgntaytty ytnwsnggng cnggngtnac nwsnggntgy 240

athytnccng cngcnmgngc nggnytnytn garytngcny tnmgngaygg nggnggngcn 300
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gtnacnytny tntggacncc ngayggngay gtnacngtnw sntggccngc ncaywsntay 420
ytnggnytng aytaygargt ncarcaymgn garwsnaayg aygaygarga ygcntggcar 480
acnacnwsng gnccntgytg ygayytnacn gtnggnggny tngayccngc nmgntgytay 540
gayttymgng tnmgngcnws nccnmgngcn gcncaytayg gnytngargc ncarccnwsn 600
gartggacng cngtnacnmg nytnwsnggn gcngcnwsng cngcnwsntg yacngcnwsn 660
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aayttycarg cntggathgc ngaygcncar gcnacngcnc cnccngcnmg nacngargar 900
gargaygayy tnathcaycc naargcnaar mgngtngarc cngargaygg nacnwsnytn 960
tgyacngtnc cnmgnccncc nwsnttygar ccnmgnggnc cnggnggngg ngcnatggtn 1020
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<220>
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<223> Oligonucleotide primer ZC694

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<400> 13
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<400> 14
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<210> 15
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   1               5                  10                  15

gtg aag gaa gcc ctg ctg cct ggt gtc ccc gac ccc cgc ggc tcc ttc      97
Val Lys Glu Ala Leu Leu Pro Gly Val Pro Asp Pro Arg Gly Ser Phe
            20                  25                  30

cct ggc ctc ttc gag aaa cat cat ggg aac ttc cag gct tgg atc gca     145
Pro Gly Leu Phe Glu Lys His His Gly Asn Phe Gln Ala Trp Ile Ala
        35                  40                  45

gat tct cag gct gct gtc cct acg gtc cca gag cag gac aaa gat gat     193
Asp Ser Gln Ala Ala Val Pro Thr Val Pro Glu Gln Asp Lys Asp Asp
    50                  55                  60

gat gtc atc cgg cct cag acc aag ggg gtg gaa act cag gag gat gat     241
Asp Val Ile Arg Pro Gln Thr Lys Gly Val Glu Thr Gln Glu Asp Asp
65                  70                  75                  80

gat gtc att gcc ccg ggg tcc cca tgc ctt ggg gga ggg gcc ctg atg     289
Asp Val Ile Ala Pro Gly Ser Pro Cys Leu Gly Gly Gly Ala Leu Met
                85                  90                  95

tcg gtg ggc ggg gcc tcg ttc ctg atg gga gac agc ggc tac acc acc     337
Ser Val Gly Gly Ala Ser Phe Leu Met Gly Asp Ser Gly Tyr Thr Thr
            100                 105                 110

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Leu  *
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<400> 16

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35 40 45
Asp Ser Gln Ala Ala Val Pro Thr Val Pro Glu Gln Asp Lys Asp Asp
50 55 60
Asp Val Ile Arg Pro Gln Thr Lys Gly Val Glu Thr Gln Glu Asp Asp
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Ser Val Gly Gly Ala Ser Phe Leu Met Gly Asp Ser Gly Tyr Thr Thr
100 105 110
Leu

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1 5

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1 5

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agc acc tct ggg ggc aca gcg gcc ctg ggc tgc ctg gtc aag gac tac 96
Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr
20 25 30

ttc ccc gaa ccg gtg acg gtg tcg tgg aac tca ggc gcc ctg acc agc 144
Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser
35 40 45

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ggc gtg cac acc ttc ccg gct gtc cta cag tcc tca gga ctc tac tcc      192
Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser
     50                  55                  60

ctc agc agc gtg gtg acc gtg ccc tcc agc agc ttg ggc acc cag acc      240
Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr
 65                  70                  75                  80

tac atc tgc aac gtg aat cac aag ccc agc aac acc aag gtg gac aag      288
Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys
                85                  90                  95

aaa gtt gag ccc aaa tct tgt gac aaa act cac aca tgc cca ccg tgc      336
Lys Val Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys
            100                 105                 110

cca gca cct gaa ctc ctg ggg gga ccg tca gtc ttc ctc ttc ccc cca      384
Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro
        115                 120                 125

aaa ccc aag gac acc ctc atg atc tcc cgg acc cct gag gtc aca tgc      432
Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys
    130                 135                 140

gtg gtg gtg gac gtg agc cac gaa gac cct gag gtc aag ttc aac tgg      480
Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp
145                 150                 155                 160

tac gtg gac ggc gtg gag gtg cat aat gcc aag aca aag ccg cgg gag      528
Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu
                165                 170                 175

gag cag tac aac agc acg tac cgt gtg gtc agc gtc ctc acc gtc ctg      576
Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu
            180                 185                 190

cac cag gac tgg ctg aat ggc aag gag tac aag tgc aag gtc tcc aac      624
His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn
        195                 200                 205

aaa gcc ctc cca gcc ccc atc gag aaa acc atc tcc aaa gcc aaa ggg      672
Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly
    210                 215                 220
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cag ccc cga gaa cca cag gtg tac acc ctg ccc cca tcc cgg gat gag      720
Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu
225                 230                 235                 240

ctg acc aag aac cag gtc agc ctg acc tgc ctg gtc aaa ggc ttc tat      768
Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr
                245                 250                 255

ccc agc gac atc gcc gtg gag tgg gag agc aat ggg cag ccg gag aac      816
Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn
            260                 265                 270

aac tac aag acc acg cct ccc gtg ctg gac tcc gac ggc tcc ttc ttc      864
Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe
        275                 280                 285

ctc tac agc aag ctc acc gtg gac aag agc agg tgg cag cag ggg aac      912
Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn
    290                 295                 300

gtc ttc tca tgc tcc gtg atg cat gag gct ctg cac aac cac tac acg      960
Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr
305                 310                 315                 320

cag aag agc ctc tcc ctg tct ccg ggt aaa                              990
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            20                  25                  30
Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser
        35                  40                  45
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Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser
    50                  55                  60
Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr
65                  70                  75                  80
Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys
                85                  90                  95
Lys Val Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys
            100                 105                 110
Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro
        115                 120                 125
Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys
    130                 135                 140
Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp
145                 150                 155                 160
Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu
                165                 170                 175
Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu
            180                 185                 190
His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn
        195                 200                 205
Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly
    210                 215                 220
Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu
225                 230                 235                 240
Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr
                245                 250                 255
Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn
            260                 265                 270
Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe
        275                 280                 285
Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn
    290                 295                 300
Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr
305                 310                 315                 320
Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
                325                 330
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<210> 30
<211> 321
<212> DNA
<213> Homo sapiens

<220>

<221> CDS
<222> (1)...(321)

<400> 30

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Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln
 1               5                  10                  15

ttg aaa tct ggt acc gcc tct gtt gtg tgc ctg ctg aat aac ttc tat       96
Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr
            20                  25                  30

ccc aga gag gcc aaa gta cag tgg aag gtg gat aac gcc ctc caa tcg      144
Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser
        35                  40                  45

ggt aac tcc cag gag agt gtc aca gag cag gac agc aag gac agc acc      192
Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr
    50                  55                  60

tac agc ctc agc agc acc ctg acg ctg agc aaa gca gac tac gag aaa      240
Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys
65                  70                  75                  80

cac aaa gtc tac gcc tgc gaa gtc acc cat cag ggc ctg agc tcg ccc      288
His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro
                85                  90                  95

gtc aca aag agc ttc aac agg gga gag tgt tag                         321
Val Thr Lys Ser Phe Asn Arg Gly Glu Cys  *
            100                 105
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<210> 31
<211> 106
<212> PRT
<213> Homo sapiens

<400> 31

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Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln
 1               5                  10                  15
Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr
            20                  25                  30
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Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser
35 40 45
Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr
50 55 60
Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys
65 70 75 80
His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro
85 90 95
Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
100 105

<210> 32
<211> 8
<212> DNA
<213> Artificial Sequence

<220>
<223> Oligonucleotide primer ZC11440

<400> 32
aattgaga 8

<210> 33
<211> 8
<212> DNA
<213> Artificial Sequence

<220>
<223> Oligonucleotide primer ZC11441

<400> 33
cgcgtctc 8

<210> 34
<211> 1306
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<213> Mus musculus

<220>
<221> CDS
<222> (74)...(1151)

<400> 34

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gcgccctcga ctcggaccgg ctcggaccga accagctgtc aatcactgca gcgtccgcgg      60
ccccgccggc gac atg gca tgg gca ctc gcg gtc atc ctc ctg cct cgg         109
               Met Ala Trp Ala Leu Ala Val Ile Leu Leu Pro Arg
                1               5                  10

ctc ctt acg gcg gca gcg gcg gcg gcg gcg gtg acg tca cgg ggt gat        157
Leu Leu Thr Ala Ala Ala Ala Ala Ala Ala Val Thr Ser Arg Gly Asp
        15                  20                  25

gtc aca gtc gtc tgc cat gac ctg gag acg gtg gag gtc acg tgg ggc        205
Val Thr Val Val Cys His Asp Leu Glu Thr Val Glu Val Thr Trp Gly
    30                  35                  40

tcg ggc ccc gac cac cac ggc gcc aac ttg agc ctg gag ttc cgt tat        253
Ser Gly Pro Asp His His Gly Ala Asn Leu Ser Leu Glu Phe Arg Tyr
45                  50                  55                  60

ggt act ggc gcc ctg caa ccc tgc ccg cga tat ttc ctg tcc ggc gct        301
Gly Thr Gly Ala Leu Gln Pro Cys Pro Arg Tyr Phe Leu Ser Gly Ala
                65                  70                  75

ggt gtc act tcc ggg tgc atc ctc ccc gcg gcg agg gcg ggg ctg ctg        349
Gly Val Thr Ser Gly Cys Ile Leu Pro Ala Ala Arg Ala Gly Leu Leu
            80                  85                  90

gag ctg gca ctg cgc gac gga ggc ggg gcc atg gtg ttt aag gct agg        397
Glu Leu Ala Leu Arg Asp Gly Gly Gly Ala Met Val Phe Lys Ala Arg
        95                  100                 105

cag cgc gcg tcc gcc tgg ctg aag ccc cgc cca cct tgg aat gtg acg        445
Gln Arg Ala Ser Ala Trp Leu Lys Pro Arg Pro Pro Trp Asn Val Thr
    110                 115                 120

ctg ctc tgg aca cca gac ggg gac gtg act gtc tcc tgg cct gcc cac        493
Leu Leu Trp Thr Pro Asp Gly Asp Val Thr Val Ser Trp Pro Ala His
125                 130                 135                 140

tcc tac ctg ggc ctg gac tac gag gtg cag cac cgg gag agc aat gac        541
Ser Tyr Leu Gly Leu Asp Tyr Glu Val Gln His Arg Glu Ser Asn Asp
                145                 150                 155
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gat gag gac gcc tgg cag acg acc tca ggg ccc tgc tgt gac ttg aca      589
Asp Glu Asp Ala Trp Gln Thr Thr Ser Gly Pro Cys Cys Asp Leu Thr
            160                 165                 170

gtg ggc ggg ctc gac ccc gcg cgc tgc tat gac ttc cgg gtt cgg gcg      637
Val Gly Gly Leu Asp Pro Ala Arg Cys Tyr Asp Phe Arg Val Arg Ala
        175                 180                 185

tcg ccc cgg gcc gcg cac tat ggc ctg gag gcg cag cct agc gag tgg      685
Ser Pro Arg Ala Ala His Tyr Gly Leu Glu Ala Gln Pro Ser Glu Trp
    190                 195                 200

aca gcg gtg aca agg ctt tcc ggg gca gca tcc gcg gcc tcc tgt acc      733
Thr Ala Val Thr Arg Leu Ser Gly Ala Ala Ser Ala Ala Ser Cys Thr
205                 210                 215                 220

gca agc ccc gcc cca tcc ccg gcc ctg gcc ccg ccc ctc ctg ccc ctg      781
Ala Ser Pro Ala Pro Ser Pro Ala Leu Ala Pro Pro Leu Leu Pro Leu
                225                 230                 235

ggc tgc ggc cta gca gcg ctg ctg aca ctg tcc ctg ctc ctg gcc gcc      829
Gly Cys Gly Leu Ala Ala Leu Leu Thr Leu Ser Leu Leu Leu Ala Ala
            240                 245                 250

ctg agg ctt cgc agg gtg aaa gat gcg ctg ctg ccc tgc gtc cct gac      877
Leu Arg Leu Arg Arg Val Lys Asp Ala Leu Leu Pro Cys Val Pro Asp
        255                 260                 265

ccc agc ggc tcc ttc cct gga ctc ttt gag aag cat cac ggg aac ttc      925
Pro Ser Gly Ser Phe Pro Gly Leu Phe Glu Lys His His Gly Asn Phe
    270                 275                 280

cag gcc tgg att gcg gac gcc cag gcc aca gcc ccg cca gcc agg acc      973
Gln Ala Trp Ile Ala Asp Ala Gln Ala Thr Ala Pro Pro Ala Arg Thr
285                 290                 295                 300

gag gag gaa gat gac ctc atc cac ccc aag gct aag agg gtg gag ccc     1021
Glu Glu Glu Asp Asp Leu Ile His Pro Lys Ala Lys Arg Val Glu Pro
                305                 310                 315

gag gac ggc acc tcc ctc tgc acc gtg cca agg cca ccc agc ttc gag     1069
Glu Asp Gly Thr Ser Leu Cys Thr Val Pro Arg Pro Pro Ser Phe Glu
            320                 325                 330
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cca agg ggg ccg gga ggc ggg gcc atg gtg tca gtg ggc ggg gcc acg     1117
Pro Arg Gly Pro Gly Gly Gly Ala Met Val Ser Val Gly Gly Ala Thr
        335                 340                 345

ttc atg gtg ggc gac agc ggc tac atg acc ctg t gaccttgaag           1161
Phe Met Val Gly Asp Ser Gly Tyr Met Thr Leu
    350                 355

tcactgccag tctatacttc aggctgaggt cacttcctgt ctttaaataa ttcaaactca  1221
caaatcctgt gcctgtctgt atgcaaatgt ggtcacgaat attcaaataa aatgcaaatg  1281
ctatgctaaa aaaaaaaaa aaaaa                                        1306
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<210> 35
<211> 359
<212> PRT
<213> Mus musculus

<400> 35

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Met Ala Trp Ala Leu Ala Val Ile Leu Leu Pro Arg Leu Leu Thr Ala
 1               5                  10                  15
Ala Ala Ala Ala Ala Ala Val Thr Ser Arg Gly Asp Val Thr Val Val
            20                  25                  30
Cys His Asp Leu Glu Thr Val Glu Val Thr Trp Gly Ser Gly Pro Asp
        35                  40                  45
His His Gly Ala Asn Leu Ser Leu Glu Phe Arg Tyr Gly Thr Gly Ala
    50                  55                  60
Leu Gln Pro Cys Pro Arg Tyr Phe Leu Ser Gly Ala Gly Val Thr Ser
65                  70                  75                  80
Gly Cys Ile Leu Pro Ala Ala Arg Ala Gly Leu Leu Glu Leu Ala Leu
                85                  90                  95
Arg Asp Gly Gly Gly Ala Met Val Phe Lys Ala Arg Gln Arg Ala Ser
            100                 105                 110
Ala Trp Leu Lys Pro Arg Pro Pro Trp Asn Val Thr Leu Leu Trp Thr
        115                 120                 125
Pro Asp Gly Asp Val Thr Val Ser Trp Pro Ala His Ser Tyr Leu Gly
    130                 135                 140
Leu Asp Tyr Glu Val Gln His Arg Glu Ser Asn Asp Asp Glu Asp Ala
145                 150                 155                 160
Trp Gln Thr Thr Ser Gly Pro Cys Cys Asp Leu Thr Val Gly Gly Leu
                165                 170                 175
Asp Pro Ala Arg Cys Tyr Asp Phe Arg Val Arg Ala Ser Pro Arg Ala
            180                 185                 190
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Ala His Tyr Gly Leu Glu Ala Gln Pro Ser Glu Trp Thr Ala Val Thr
        195                 200                 205
Arg Leu Ser Gly Ala Ala Ser Ala Ala Ser Cys Thr Ala Ser Pro Ala
    210                 215                 220
Pro Ser Pro Ala Leu Ala Pro Pro Leu Leu Pro Leu Gly Cys Gly Leu
225                 230                 235                 240
Ala Ala Leu Leu Thr Leu Ser Leu Leu Leu Ala Ala Leu Arg Leu Arg
                245                 250                 255
Arg Val Lys Asp Ala Leu Leu Pro Cys Val Pro Asp Pro Ser Gly Ser
            260                 265                 270
Phe Pro Gly Leu Phe Glu Lys His His Gly Asn Phe Gln Ala Trp Ile
        275                 280                 285
Ala Asp Ala Gln Ala Thr Ala Pro Pro Ala Arg Thr Glu Glu Glu Asp
    290                 295                 300
Asp Leu Ile His Pro Lys Ala Lys Arg Val Glu Pro Glu Asp Gly Thr
305                 310                 315                 320
Ser Leu Cys Thr Val Pro Arg Pro Pro Ser Phe Glu Pro Arg Gly Pro
                325                 330                 335
Gly Gly Gly Ala Met Val Ser Val Gly Gly Ala Thr Phe Met Val Gly
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Asp Ser Gly Tyr Met Thr Leu
        355
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<210> 36
<211> 61
<212> DNA
<213> Artificial Sequence

<220>
<223> Section of human kappa light chain including KpnI site to be used in oligonucleotide primer construction

<400> 36
actgtggctg caccatctgt cttcatcttc ccgccatctg atgagcagtt gaaatctggt a 60
61

<210> 37
<211> 100
<212> DNA
<213> Artificial Sequence

<220>

<223> Oligonucleotide primer ZC12749

<400> 37
gtaccttccc gtaaatccct ccccttcccg gaattacacc cgcgtatttc ccagaaaagg 60
aactgtagat ttctaggaat tcaatccttg gccacgcgtc 100

<210> 38
<211> 100
<212> DNA
<213> Artificial Sequence

<220>
<223> Oligonucleotide primer ZC12748

<400> 38
tcgagacgcg tggccaagga ttgaattcct agaaatctac agttcctttt ctgggaaata 60
cgcgggtgta attccgggaa ggggagggat ttacgggaag 100

<210> 39
<211> 1077
<212> DNA
<213> Artificial Sequence

<220>
<223> Degenerate polynucleotide seqence of zcytor10
polypeptide of SEQ ID NO:35

<221> misc_feature
<222> (1)...(1077)
<223> n = A,T,C or G

<400> 39
atggcntggg cnytngcngt nathytnytn ccnmgnytny tnacngcngc ngcngcngcn 60
gcngcngtna cnwsnmgngg ngaygtnacn gtngtntgyc aygayytnga racngtngar 120
gtnacntggg gnwsnggncc ngaycaycay ggngcnaayy tnwsnytnga rttymgntay 180
ggnacnggng cnytncarcc ntgyccnmgn tayttyytnw snggngcngg ngtnacnwsn 240
ggntgyathy tnccngcngc nmgngcnggn ytnytngary tngcnytnmg ngayggnggn 300
ggngcnatgg tnttyaargc nmgncarmgn gcnwsngcnt ggytnaarcc nmgnccnccn 360
tggaaygtna cnytnytntg gacnccngay ggngaygtna cngtnwsntg gccngcncay 420
wsntayytng gnytngayta ygargtncar caymgngarw snaaygayga ygargaygcn 480
tggcaracna cnwsnggncc ntgytgygay ytnacngtng gnggnytnga yccngcnmgn 540
tgytaygayt tymgngtnmg ngcnwsnccn mgngcngcnc aytayggnyt ngargcncar 600
ccnwsngart ggacngcngt nacnmgnytn wsnggngcng cnwsngcngc nwsntgyacn 660

gcnwsnccng cnccnwsncc ngcnytngcn ccnccnytny tnccnytngg ntgyggnytn 720
gcngcnytny tnacnytnws nytnytnytn gcngcnytnm gnytnmgnmg ngtnaargay 780
gcnytnytnc cntgygtncc ngayccnwsn ggnwsnttyc cnggnytntt ygaraarcay 840
cayggnaayt tycargcntg gathgcngay gcncargcna cngcnccncc ngcnmgnacn 900
gargargarg aygayytnat hcayccnaar gcnaarmgng tngarccnga rgayggnacn 960
wsnytntgya cngtnccnmg nccnccnwsn ttygarccnm gnggnccngg nggnggngcn 1020
atggtnwsng tnggnggngc nacnttyatg gtnggngayw snggntayat gacnytn 1077

<210> 40
<211> 5
<212> PRT
<213> Homo sapiens

<400> 40
Pro Ser Trp Glu Thr
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<210> 41
<211> 32
<212> PRT
<213> Artificial Sequence

<220>
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<221> VARIANT
<222> (1)...(32)
<223> Xaa = Any Amino Acid

<400> 41
Arg Val Lys Xaa Xaa Leu Xaa Pro Xaa Val Pro Asp Pro Xaa Xaa Xaa
1 5 10 15
Phe Pro Gly Leu Phe Glu Xaa His Xaa Gly Asn Phe Gln Xaa Trp Ile
20 25 30

<210> 42
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<221> VARIANT
<222> (2)...(2)

<223> Xaa = Leu or Ile

<221> VARIANT
<222> (4)...(4)
<223> Xaa = Cys, Gly, or Ser

<223> Consensus Box I polypeptide motif

<400> 42
Leu Xaa Pro Xaa Val Pro Asp Pro
1 5

<210> 43
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<221> VARIANT
<222> (1)...(8)
<223> Xaa = Any Amino Acid

<223> Polypeptide motif, Motif 1

<400> 43
Leu Glu Thr Val Xaa Val Thr Trp
1 5

<210> 44
<211> 4
<212> PRT
<213> Artificial Sequence

<220>
<223> Polypeptide motif, Motif 2

<400> 44
Thr Ser Gly Cys
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<210> 45
<211> 4
<212> PRT

<213> Artificial Sequence

<220>
<223> Polypeptide motif, Motif 5

<400> 45
Tyr Glu Val Gln
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INTERNATIONAL SEARCH REPORT

In tional Application No
PCT/US 00/12924

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N15/12 C12N15/62 C07K14/715 C07K16/28 C12Q1/68
G01N33/68

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C07K C12N C12Q G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
STRAND, EPO-Internal, EMBL

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DATABASE EMBL 'Online! EBI; ACC. NO.: AA018020, 10 August 1996 (1996-08-10) MARRA ET AL.: "The WashU-HHMI Mouse EST project" XP002147793 the whole document ---	1-12, 17-24
X	DATABASE EMBL 'Online! EBI; ACC. NO.: AA008678, 28 July 1996 (1996-07-28) MARRA ET AL.: "The WashU-HHMI Mouse EST Project" XP002147794 the whole document --- -/--	1-12, 17-24

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
25 September 2000	02/10/2000

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INTERNATIONAL SEARCH REPORT

Ir ational Application No
PCT/US 00/12924

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
A	BAZAN J F: "Structural design and molecular evolution of a cytokine receptor superfamily" JOURNAL OF BIOLOGICAL CHEMISTRY,AMERICAN SOCIETY OF BIOLOGICAL CHEMISTS, BALTIMORE, MD,US, vol. 87, September 1990 (1990-09), pages 6934-6938, XP002111161 ISSN: 0021-9258 figure 1	1-24
A	MCKINNON M ET AL: "STRATEGIES FOR THE DISCOVERY OF CYTOKINE RECEPTOR ANTAGONISTS" DRUG NEWS AND PERSPECTIVES,XX,XX, vol. 9, 1996, pages 389-398, XP000882849 ISSN: 0214-0934 page 392, column 3 -page 396, column 2	29-32
A	US 5 747 292 A (NELSON BRAD H ET AL) 5 May 1998 (1998-05-05) claims 1-31; figures 1,2	13-16
P,X	FUJIO ET AL.: "Molecular cloning of a novel type 1 cytokine receptor similar to the common gamma chain" BLOOD, vol. 95, no. 7, 1 April 2000 (2000-04-01), pages 2204-2211, XP000946169 figures 1,2,4	1-24
E	WO 00 49148 A (FUJIO KEISHI ;KITAMURA TOSHIO (JP)) 24 August 2000 (2000-08-24) figures 1,5,6	1-32

INTERNATIONAL SEARCH REPORT

Information on patent family members

In tional Application No
PCT/US 00/12924

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 5747292 A	05-05-1998	AU 695869 B	27-08-1998
		AU 6627194 A	24-10-1994
		CA 2160011 A	13-10-1994
		EP 0693084 A	24-01-1996
		JP 8511000 T	19-11-1996
		WO 9422914 A	13-10-1994
WO 0049148 A	24-08-2000	NONE	